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COPPER AND SILVER IONS MORE EFFECTIVE AGAINST LEGIONELLAE THAN AGAINST MYCOBACTERIA IN A HOSPITAL WARM WATER SYSTEM

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Abstract—We studied the influence of electrolytically released copper and silver ions on the microbiological quality in a warm water system of a hospital. The concentration of nontuberculous mycobacteria was followed for three, and that of legionellae and other heterotrophic bacteria in the water for four years. The highest concentrations of copper and silver ions were 220 and 68 μg/l, respectively. Silver ion concentration of about 3 μg/l was sufficient to control the growth of legionellae in circulating warm water. The results showed that it is more difficult to eradicate legionellae from taps and showers: these points were colonized by a small number of legionellae after the metal ion concentrations were increased in the circulating water. A regular use of water eradicated legionellae from the shower. One tap was still used irregularly, and this may be a reason why it still contained small concentrations of legionellae also in the last years of the study. Mycobacteria were occasionally isolated from the circulating water and repeatedly from the shower, even when the metal concentrations were high. To control legionella bacteria in warm water systems, silver concentrations of only 3 μg/l are needed if all taps and showers of the system are regularly used. Such low copper and silver concentrations, however, are not efficient against nontuberculous mycobacteria or other heterotrophic bacteria.

Key words—copper, silver, ionisation, prevention, control, legionella, mycobacteria

INTRODUCTION

Legionellae and nontuberculous mycobacteria are common heterotrophic bacteria in water distribution systems. These bacteria are a problem especially in water systems of hospitals, where they may cause nosocomial infections, usually in patients with other underlying diseases (Edelstein, 1988; Wallace *et al.*, 1998). A severe nosocomial Legionnaires' disease case occurred in a 460-bed hospital caused by *Legionella pneumophila* serogroup 6, and the probable source of infection was a warm water system. The concentrations of legionella bacteria detected in water samples varied from 3.5×10^2 to 2.0×10^4 cfu/l. Warm water systems were cleaned with flushing 75°C water twice for 30 min, and all tanks of warm water were removed. The temperature of the water leaving from heat exchanger was set to 60°C. Control samples, however, still contained high concentrations of legionellae. Also many previous studies have shown the difficulties of thermal eradication in large water systems (Fischer-Hoch *et al.*, 1984; Muraca *et al.*, 1990).

Several species of nontuberculous mycobacteria may cause nosocomial infections. Most cases are local wound infections or abscesses caused by rapidly growing species (Wallace *et al.*, 1998). In immunocompromised patients, the infections may be disseminated. The occurrence of mycobacteria in hospital water systems has been known for a long time (Bailey *et al.*, 1970; Bullin *et al.*, 1970; McSwiggan and Collins, 1974). With the recent molecular techniques the hospital water supplies have been able to verify as the source of mycobacterial infections acquired in hospitals (von Reyn *et al.*, 1994; Kauppinen *et al.*, 1999). Resistance of mycobacteria to disinfectants and high temperatures (Carson *et al.*, 1978; Merkal and Crawford, 1979; Best *et al.*, 1988; Schulze-Röbbecke and Buchholtz, 1992) makes them extremely difficult to eradicate from tap water. Chlorination (Lockwood *et al.*, 1989) and temporary heating of the hot water combined with flushing of faucets and shower heads (Sniadick *et al.*, 1993) have been used to control mycobacteria

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in hospital water systems. Long-term efficacy of these preventive measures against mycobacteria is not known.

Copper and silver ions electrolytically released in water is a new and promising prevention method against legionella bacteria (Liu *et al.*, 1994). Silver ions inhibit bacterial growth by interfering with electron transport, binding DNA and interacting with cell membrane (Tilton and Rosenberg, 1978). Copper ions may function by enhancing displacement reactions, disrupting enzyme structure and binding thiol or other groups on protein molecules (Thurman and Gerba, 1989). Landeen *et al.* (1989) have reported enhanced inactivation of legionella bacteria when 400 µg/l of copper, 40 µg/l of silver and 0.4 mg/l of chlorine were combined in their *in vitro* experiments. Legionellae have been eliminated from a hospital water distribution system *in situ* at copper/silver concentrations of 400/40 µg/l or greater (Liu *et al.*, 1994). More than 30 hospitals in USA are using copper–silver ionisation to control the growth of legionellae in their water systems (Lin *et al.*, 1998a). A laboratory study has suggested that mycobacteria could also be eliminated from water systems by copper and silver ions (Lin *et al.*, 1998b), but there are no field studies relating to this matter.

In Finland, the technical and aesthetic limit concentration for copper in drinking water is 1000 µg/l and that for silver is 10 µg/l (Anon, 1994). The aim of this study was to investigate if legionellae, nontuberculous mycobacteria and other heterotrophic bacteria could be eliminated from a warm water system of a Finnish hospital by low concentrations of copper and silver ions.

MATERIALS AND METHODS

Water treatment system

A water treatment unit (TPU4-4/3, Tarn-Pure System, Pan-Ionic Ltd., UK) was installed in a warm water system of a hospital, at the site where warm water returns to the heat exchanger (Fig. 1). Copper and silver ions were continuously released in the water by the treatment unit, which could handle 4.5 m^3 water in a day. The daily warm water consumption of the system was 4 m^3. The copper–silver electrodes contained 70% of copper and 30% of silver. Before installation the original plastic cover of ionisation cell was replaced with steel cover. The electrodes remained attached to the original plastic tube.

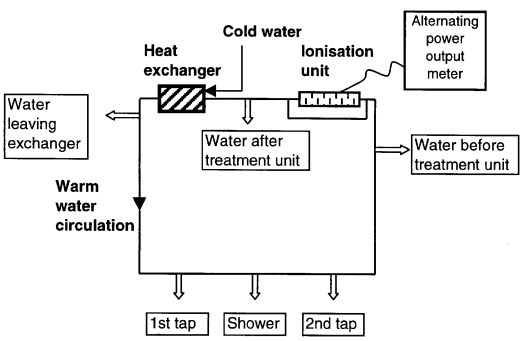

Fig. 1. Location of the ionisation unit and the sampling sites in the warm water system.

Sampling

Water samples were taken 2 months, 1 month and 2 weeks before the treatment began. The output current of the unit was slowly increased and since the 8th sampling time it was held 350 mA. The samples were taken at sites where water was leaving from and returning to the heat exchanger in the warm water circulation. The returning water was sampled both before and after copper–silver ionisation unit. From the peripheral sites, the warm water samples were taken from two taps and a shower. Cold water feeding the warm water system was also studied. The samples from peripheral sites were taken without flushing, the other samples were taken after flushing until water temperature had reached it highest or lowest (cold water) value. Samplings were made 5 days, 2 weeks, 1 month, 2, 4, 5, 6, 7 and 11 months, 1.2, 1.4, 2.1, 2.5, 3.1 and 3.5 years after the ionisation unit was turned on. After the 8th sampling time, to ensure a more regular use of shower, water from shower was flushed for 5 min twice a week. The electrodes were replaced with the ones before the last samples. In the 4th year samples taken only from warm water before and after ionisation unit, taps and shower were analysed for legionella, heterotrophic bacteria and metal ion concentrations.

The samples for bacterial analyses were taken in sterile polypropylene bottles with added sodium thiosulphate for removing residual free chlorine in water (ISO, 1998). The taps and a shower head were wiped with a sterile cotton swab, which was put to 1/40 Ringers solution for legionella analyses (ISO, 1998). The samples for metal ion analyses were taken to acid washed glass bottles and the samples for chlorine analyses to glass bottles with ground-glass stoppers.

Bacterial analyses

For culture of legionella samples, both a selective medium (MWY; Wadowsky and Yee, 1981; Edelstein, 1982) and a non-selective medium (BCYEα; Edelstein, 1981) were used. One liter water samples were concentrated by membrane filtration (Ultipor®, NR047100, pore size 0.2 µm, Pall Corp., UK). The filter was cut into small pieces and shaken in a mixer (Vortex genie 2, Scientific Industries Inc.) for 5 min with 5 ml of original sample water and glass beads. Samples of 100 µl of this concentrated water were used to inoculate MWY and BCYEα media. For decontamination of other microbes, concentrated sample water was treated with acid wash pretreatment (HCl–KCl-solution, pH 2.2, 4 min; Bopp *et al.*, 1981), or heat pretreatment (50°C, 30 min, Dennis *et al.*, 1984). A portion of 0.1 ml of the unconcentrated sample water was directly inoculated on MWY. Inoculated media were incubated at 37°C and the growth was checked 3 times weekly during 3 weeks. Legionella strains were identified with growth tests and direct fluorescence antibody-test (Sanofi Diagnostic Pasteur). The lowest detection limit of legionellae was 50 cfu/l in the first eight samplings. Later, the sensitivity was increased to 5 cfu/l by centrifugation (6000 *g*, 10 min, Biofuge A, Heraues Christ) following the first concentration by filtration. The occurrence of legionellae in taps and a shower head wiped with cotton swabs was determined with MWY medium with and without acid wash pretreatment. Legionella analyses were made through the whole 4 years experiment.

For mycobacterial cultures, the microbes were concentrated from one to two l of water by membrane filtration (Ultipor®, NR047100, Pall Corp., UK) and eluted from the filter as described earlier (Iivanainen *et al.*, 1993). In the first

year, two 5-ml concentrates were made from every sample. The first was decontaminated with cetylpyridinium chloride (0.005%, 45 min) and the other with NaOH (0.7 M, 35 min) and oxalic acid (5%, 35 min). The exposure times included 15 min centrifugation times (8600 *g*, Sorvall RC-5C, Du Pont Company, Wilmington, DE). After decontamination the sediments were washed with 30 ml of sterile deionized water and the sediment resuspended in 400 µl of sterile deionized water. In the next years, only one concentrate was made of each sample for the decontamination with cetylpyridinium chloride. The exposure time was decreased to 20 min in the last year. Egg media supplemented with cycloheximide (500 µg/ml) and glycerol (pH 6.3) or Na-pyruvate (pH 6.3) were used. Samples of 50 µl of the nondecontaminated concentrates and the resuspended decontaminated sediments were inoculated onto slopes. Cycloheximide was omitted from the media after the first year. In the first and last years, additional slopes of pH 5.5 were also inoculated. The cultures were incubated at 30°C, in the last year also at 35°C, for at least 3 months. The detection limits for the analyses of the nondecontaminated samples were from 30 to 150 cfu/l and for the decontaminated samples from 2 to 10 cfu/l. Acid-fastness of the colonies was checked by Ziehl–Neelsen staining. The acid-fast strains were analysed for fatty acid and alcohol composition by gas liquid chromatography (Jantzen *et al*., 1989; Torkko *et al*., 1998) and tested for growth characteristics and selected biochemical tests. The isolates with the fatty acid profile of *M. avium-intracellulare* were also tested with *M. avium* complex probe (Gen-Probe, San Diego, CA). Mycobacteria were analysed during the first 3 years of the follow-up from the cold water, the circulating water and the shower water. Some samples of warm water leaving from exchanger also were studied for mycobacteria.

For determining viable counts of heterotrophic bacteria a 10-fold dilution series was made and aliquots of 0.1 ml of the diluted sample were used to inoculate R2A agar (Reasoner and Geldreich, 1985; Difco, Detroit, MI). The samples were incubated at 20°C (mesophilic bacteria) and at 50°C (thermophilic bacteria) for 4 weeks. The lowest detection limit of heterotrophic bacteria was 10^4 cfu/l.

Metal ions, chlorine, pH and water temperature

Acidified samples (1.0% HNO_3) for copper and silver were concentrated by boiling and then analysed by an atomic absorption spectrophotometer (Perkin-Elmer 4100, Outokumpu Research). Free and total chlorine concentrations were analysed with a titrimetric method (Finnish standard, SFS 3004). pH values were measured with pH meter (Orion Research, digital pH/millivolt meter 611). Water temperatures were measured with a multimeter (DAN 1200 Super, Landis & Gyr, Bilman A-S) after samples were taken.

Statistical analyses

Wilcoxon matched-pairs signed-ranks test was used to compare the concentrations of metals and bacteria in the beginning (the first 7 sampling times) and the end (the last 11 times) of the experiment.

RESULTS

Metal concentrations

Before the use of the treatment unit (the first 3 sampling times) copper concentrations in the warm water varied from 37 to 110 µg/l and silver concentrations from 0 to 1 µg/l. There was an increase in the silver concentrations at all warm water sampling sites after the 7th samples when the output current of the unit was raised to 350 mA. The silver concentrations, however, decreased at the end of the 2nd year because of build-up of scale on electrodes. Since then the electrodes were cleaned every 3rd month. The copper concentrations did not increase at the beginning of the experiment and did not decrease because of scale on electrodes as much as the silver concentrations did. In the 3rd year the electrodes were moved closer to each other because they had become worn off. The maximal concentrations of metals, 220 µg/l of copper and 68 µg/l of silver, were reached in the 4th year. During the end part of the experiment the mean metal concentrations at the different sampling sites of the warm water system varied from 86 to 150 µg Cu/l and from 3 to 11 µg Ag/l (Table 1). There were statistically significant increases in both copper and silver concentrations in the warm water system as a result of the treatment.

Concentrations of legionellae and mycobacteria

In the cold water, viable counts of legionellae were always below the detection limit (Fig. 2), whereas mycobacteria were repeatedly isolated from these samples. In the warm water just leaving the heat exchanger neither viable legionella bacteria nor mycobacteria were detected.

The other sampling sites, taps, shower and warm water returning back to heat exchanger, were contaminated with legionellae at the beginning of the experiment (Figs 3–6). The highest legionella concentration, 1.1×10^7 cfu/l, was in the first sample from the shower. Before the treatment, mycobacteria were occasionally detected from the water returning to exchanger (50 cfu/l, Fig. 3) and repeatedly from the shower water with concentration up to 1400 cfu/l (Fig. 6).

After the mean silver concentration increased over 3 µg/l, legionellae disappeared from the circulating warm water but mycobacteria were still occasionally detected (Fig. 3). The circulating water remained free from legionellae until the mean silver concentration fell below 3 µg/l because of scale formation on electrodes. Since then, regular cleaning of the electrodes kept the silver concentrations higher in circulating water, where legionellae were not detected for the last 2 years. The last two samples for mycobacteria taken in the 3rd year did not contain mycobacteria.

Legionellae were isolated from the first tap only twice (Fig. 4). Silver concentrations were then below 2 µg/l. The maximum copper concentration reached at this tap was 170 µg Cu/l. Cotton swab samples from this tap in regular use never contained legionella bacteria. Legionellae were isolated from the second tap (Fig. 5), even though the copper and silver concentrations were high (Cu from 99 to 220 µg/l, Ag from 4 to 54 µg/l). These concentrations, however, were much lower (only up 50 cfu/l) than before the copper–silver treatment (up to

Table 1. Mean, minimum and maximum concentrations of metal ions and microbes at the beginning (to the 7th samples) and the end part of the experiment (from the 8th samples), and the statistical significance of the differences[a] between these means

Water parameter and site of sampling	Concentrations at the beginning			Concentrations at the end			Statistical significance
	Mean	Range	*n*	Mean	Range	*n*	
Copper (μg/l), all sites	**84**	**29–150**	**42**	**120**	**0.7–220**	**64**	
Water leaving exchanger	74	69–90	7	86	0.7–130	9	
Water before treatment unit	97	87–110	7	130	110–170	11	
Water after treatment unit	73	29–120	7	120	96–150	11	$P<0.05$
1st tap	82	61–99	7	120	83–170	11	
2nd tap	110	85–150	7	150	99–220	11	
Shower	70	59–84	7	97	67–150	11	
Silver (μg/l), all sites	**0.3**	**0–1.7**	**42**	**7.0**	**0–68**	**64**	
Water leaving exchanger	0	0	7	3.1	0–11	9	
Water before treatment unit	0.1	0–0.9	7	11	1.9–68	11	
Water after treatment unit	0.2	0–1.0	7	6.3	1.4–14	11	$P<0.05$
1st tap	0.8	0–1.7	7	7.1	2.5–27	11	
2nd tap	0.2	0–0.7	7	9.8	0–54	11	
Shower	0.5	0–1.0	7	4.1	0.6–14	11	
Legionella (cfu/l), all sites	**2.7×10^5**	**$0–1.1\times10^7$**	**42**	**2800**	**$0–1.8\times10^5$**	**64**	
Water leaving exchanger	0	0	7	0	0	9	
Water before treatment unit	110	0–500	7	4.5	0–50	11	
Water after treatment unit	770	0–5100	7	0	0	11	$P<0.05$
1st tap	1500	$0–1.0\times10^4$	7	0	0	11	
2nd tap	4400	$0–3.0\times10^4$	7	8.5	0–50	11	
Shower	1.6×10^6	$0–1.1\times10^7$	7	1.6×10^4	$0–1.8\times10^5$	11	
Mycobacteria (cfu/l), all sites	**160**	**0–1400**	**21**	**70**	**0–1200**	**28**	
Water leaving exchanger	ND[b]	ND[b]	0	0	0	5	
Water before treatment unit	7.1	0–50	7	0	0	7	
Water after treatment unit	7.1	0–50	7	15	0–75	7	
Shower	480	100–1400	7	210	0–1200	9	
Mesophilic heterotrophic bacteria (cfu/l), all sites	**1.6×10^8**	**$0–2.0\times10^9$**	**42**	**5.7×10^7**	**$0–1.1\times10^9$**	**64**	
Thermophilic heterotrophic bacteria (cfu/l), all sites	**3.0×10^7**	**$0–2.6\times10^8$**	**42**	**1.4×10^7**	**$0–1.4\times10^8$**	**64**	

[a] Wilcoxon matched-pairs signed-ranks test.
[b] ND, not determined.

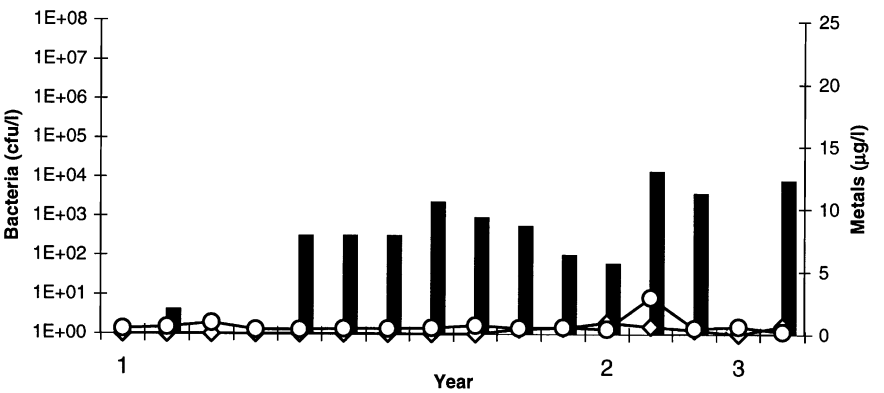

Fig. 2. Concentrations of mycobacteria (black columns), copper (○) and silver (◇) in the cold water. Copper concentration in figure should be multiplied by 10 to reach the real copper level. Legionella bacteria were not found from cold water samples.

3.0×10^4 cfu/l). At the beginning of the experiment also the cotton swab samples contained legionella bacteria. This tap was located in an examination room and was used irregularly.

Despite of high output current of the copper–silver unit, only minor increases in the copper and silver concentrations were detected in the shower water at the beginning of the experiment, and also the legionella concentration remained high (Fig. 6). Also the cotton swab samples contained legionella bacteria. The shower was used very irregularly until warm water flushings (5 min twice a week) were started after the 8th samples. As a result of these weekly flushings, the silver concentration in the shower

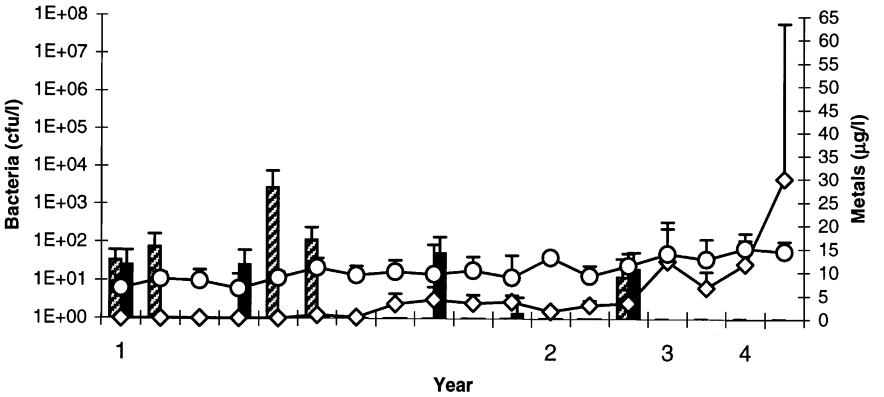

Fig. 3. Mean concentrations and standard deviations of legionellae (hatched columns), mycobacteria (black columns), copper (○) and silver (◇) in the circulating warm water. Samples were taken from water leaving from and returning to heat exchanger before and after the ionisation unit. In the fourth year mycobacteria were not studied from circulating warm water. Copper concentration in figure should be multiplied by 10 to reach the real copper level.

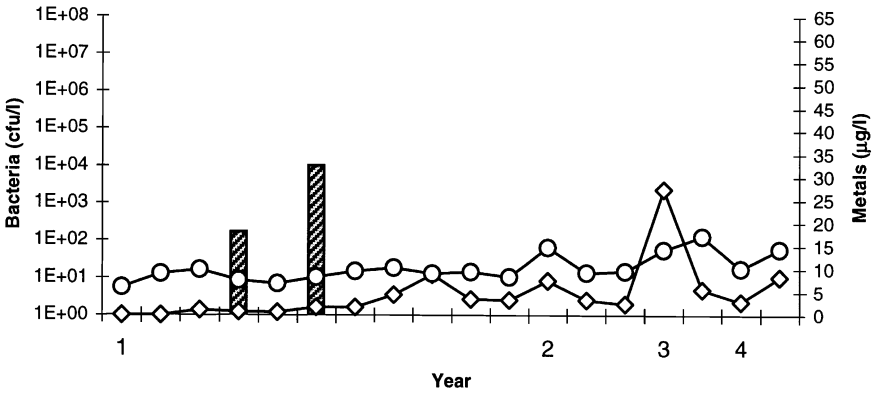

Fig. 4. Concentrations of legionellae (hatched columns), copper (○) and silver (◇) in the samples of the first tap. Mycobacteria were not analysed from these samples. Copper concentration in figure should be multiplied by 10 to reach the real copper level.

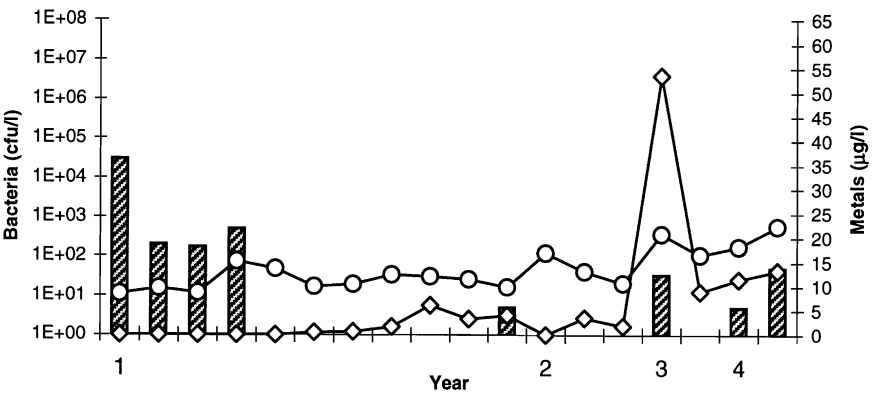

Fig. 5. Concentrations of legionellae (hatched columns), copper (○) and silver (◇) in the samples of the second tap. Mycobacteria were not analysed from these samples. Copper concentration in figure should be multiplied by 10 to reach the real copper level.

water exceeded 2 µg/l and legionellae were not detected. Simultaneous increase of copper concentration was not noticed.

When the silver concentration decreased below 2 µg/l due to scale on electrodes, legionellae were again isolated from the shower water. During the last five samplings in the 3rd and 4th year the shower water did not contain viable legionellae. At that time the copper concentration varied from 96 to 150 µg/l and that of silver from 3 to 14 µg/l. Mycobacteria

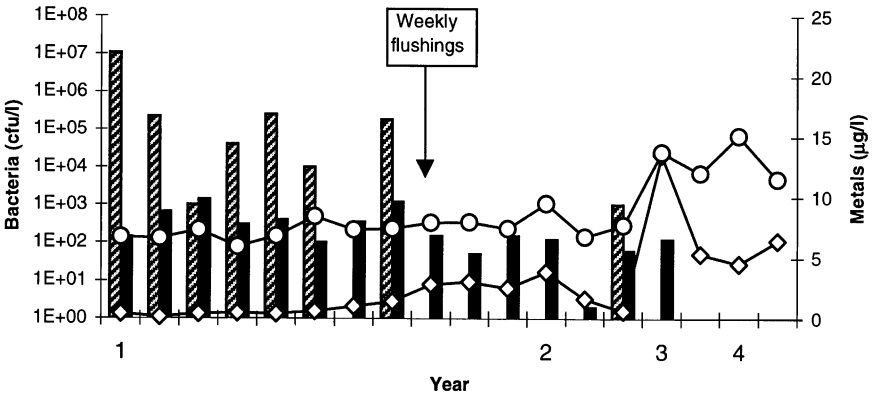

Fig. 6. Concentrations of legionellae (hatched columns), mycobacteria (black columns), copper (○) and silver (◇) in the shower water. In the fourth year mycobacteria were not studied from shower water. Copper concentration in figure should be multiplied by 10 to reach the real copper level.

were isolated from 15 of the 16 shower water samples taken during 3 years despite of the high copper and silver concentrations achieved (up to 140 and 14 µg/l, respectively, Fig. 6) and the regular flushings of this sampling site.

In general, the mean legionella concentrations in the warm water were lower at the end than in the beginning of the experiment (Table 1, $P<0.05$). The difference in mycobacterial concentrations was not statistically significant.

All legionella isolates identified were *Legionella pneumophila* species. The mycobacterial isolates were divided into six groups based on their fatty acid and alcohol composition. Three groups consisted of rapidly growing and three of slowly growing species. One group was identified as *M. gordonae*, the rest represented species which are not usually found from Finnish patients and they were not identified more closely. The rapid growers were only detected in the incoming cold water. *M. gordonae* was isolated only from the warm water at several sites, the other slow growers from both the incoming cold water and from warm waters at several sites.

Other bacterial counts

The viable counts of the mesophilic and thermophilic heterotrophic bacteria decreased only at three sampling sites of the six sites studied and the differences in these mean concentrations between the end and the beginning were statistically insignificant (Table 1).

Chlorine, pH and temperature

Free chlorine was not detected in the water. In the warm water the total chlorine concentrations were generally below the detection limit. However, in a few warm water samples the total chlorine concentration was 0.1 mg/l. In the cold water the maximum total chlorine concentration was 0.2 mg/l. The mean pH value in the cold water samples was 7.5 (range, 7.1–8.1) and in the warm water 7.6 (range, 7.2–8.2). The mean water temperature was 65°C (range, 57–74°C) in the circulating warm water leaving the exchanger and 52°C (range, 35–59°C) in the returning water. At the peripheral sites the mean warm water temperature was 54°C (range, 47–63°C). The mean cold water temperature was 8°C (range, 2–17°C).

DISCUSSION

The achieved copper and, especially, silver concentrations lowered the legionella counts at every previously legionella-positive sampling site. In the circulating warm water, silver ion concentration higher than 3 µg/l diminished legionella content to undetectable. The results of the shower show that also at peripheral sites such a low silver concentration would be high enough to eradicate legionellae, if the site is used regularly. However, when there was no regular use, even a high silver concentration in water did not totally prevent the growth of legionellae (Fig. 5). The importance of regular use of all points in the water system has also been noticed in a previous study of Liu *et al.* (1994).

Nontuberculous mycobacteria were not controlled in the system by copper and silver concentrations efficient against legionellae. In the circulating warm water, isolation frequency of legionellae decreased at the end part of the study but mycobacteria were isolated as frequently as at the beginning. In the shower water, with the concentrations up to 140 µg Cu/l and 14 µg Ag/l during the first 3 years when mycobacteria were studied, we saw a clear decrease in legionella but not in mycobacterial counts. Our results are consistent with those by Lin *et al.* (1998b) who showed *in vitro* that *Mycobacterium avium* is more tolerant to copper and silver ions than legionellae. They also showed that *M. avium* is sensitive to copper and silver concentrations of 100 and 10 µg/l, respectively, or higher (Lin *et al.*, 1998b). Our results show that mycobacteria were not affected

in situ by copper/silver concentrations at the level 100/10 μg/l and suggest that higher concentrations would be needed to eradicate mycobacteria from water systems. Mycobacteria in our waters were species other than used by Lin *et al.* (1998b). They, however, did not find differences in the sensitivity of different species of mycobacteria to copper and silver ions at the concentrations tested (Cu/Ag, 200/20 μg/l or higher) (Lin *et al.*, 1998b).

In our study, also the preventive effect against the growth of mesophilic or thermophilic heterotrophic bacteria was poor. According to States *et al.* (1998), even much higher metal concentrations have not destroyed other non-*Legionellaceae* bacteria. Amoebae, the natural hosts of legionellae (Rowbotham, 1984), have not been controlled successfully by the copper–silver device (States *et al.*, 1998). The control of amoebae seems to necessitate the simultaneous use of 800 μg Cu/l, 80 μg Ag/l and 1 mg Cl_2/l (Cassells *et al.*, 1995). Thus, the copper–silver ionisation may allow legionellae to multiply inside protozoa, but legionella cells released to free water may be destroyed by the metal ions.

A significant decrease in legionella colonization, even a total removal from peripheral sites of warm water systems, has been reached in many previous studies with higher metal concentrations. In a low water volume (<200 l) system, Liu *et al.* (1994) successfully used concentrations of 400 μg Cu/l and 40 μg Ag/l, lower ion concentrations did not eliminate legionellae from distal sites. In high water volume systems (10–30 m^3), Mietzner *et al.* (1997) used concentrations up to 140 μg Ag/l with a good results of prevention. States *et al.* (1998) accidently used even higher concentrations (mean, 711 μg Cu/l, 909 μg Ag/l). These high metal concentrations did not totally eradicate legionellae from their system, although legionella concentrations decreased. In that study, the water system had a very low warm water temperature, 39°C, and there was no information how regularly these sampling sites were used. Copper and silver ions may be more efficient against legionellae in a warm water system of high water temperature than in those with low temperature (Rohr *et al.*, 1996). The British authorities have also concluded that the metal ionisation method (400 μg Cu/l and 40 μg Ag/l) is effective against legionellae in warm water systems (Anon, 1998). They recommend the use of this method or the chlorine dioxide disinfection, if water temperature high enough to prevent growth of legionellae cannot be achieved. Our results show that for controlling the growth of legionellae even at peripheral distal sites, more important than huge concentration of metal ions is the regular use of all taps and showers. The difference in water quality and system design, however, must be taken into consideration, higher metal concentrations may be needed in some warm water systems to control the growth of legionellae (Liu *et al.*, 1998).

Free chlorine was not detected in our water samples, and also the concentrations of combined chlorine did not exceed 0.1 mg/l in warm water. Thus, the preventive effect against legionellae was not due to chlorine. *In vitro* study of Landeen *et al.* (1989) showed that chlorine increases the preventive effect of metals. Compared to other growth prevention methods, heat-and-flush, and instantaneous steam heater and chlorination, copper–silver ionisation has controlled better the growth of legionellae in warm water systems (Colville *et al.*, 1993, Mietzner *et al.*, 1997; Stout *et al.*, 1998). Our study also showed that the copper–silver ionisation method can control the growth of legionellae in warm water system if previous heat-and-flush method and permanent increase in temperature of warm water had failed. Temporary water temperature increase and flushings may be reasonable to perform in each system before the copper–silver treatment is used, to remove most of the biofilm from the system.

The results of the shower and the circulating water, where legionella concentration decreased with the increase in the silver and without increase in the copper concentration, suggest that silver was a more efficient metal ion against legionellae than copper. In the previous *in situ* study of Liu *et al.* (1994), the increase in copper concentration from 50 to 200 μg/l did not decrease the occurrence of legionellae, when silver concentration remained at 1 μg/l. Thus, it may be possible that the low copper concentrations in our study (up to 220 μg/l) had minor effect on legionellae, and it was merely silver ions acting against these bacteria. This is also supported by Rohr *et al.* (1996), who in an *in vitro* study found silver (20 μg Ag/l) more efficient against legionellae than copper (1000 μg Cu/l). However, both copper and silver concentrations increased during our experiment and it is therefore difficult to conclude the relative importance of these ions against legionellae. Compared to silver, copper seem to penetrate better into biofilm (Liu *et al.*, 1998). In study of Lin *et al.* (1996) copper (100 μg/l) was found to be more efficient than silver (80 μg/l) against legionellae. It has been observed that the concentration of copper ions correlated negatively with the number of legionellae in warm water systems (Zacheus and Martikainen, 1994). As a synergistic effect of copper and silver ions has been detected (Lin *et al.*, 1996), both metals may have an important role in the prevention of the bacterial growth.

Silver concentrations higher than the technical and aesthetic limit value in Finland, 10 μg/l (Anon, 1994), were detected in 13 samples of our study. Such concentrations have not, however, been harmful because even higher levels of silver, up to 100 μg/l, could be used in drinking water treatment without risk to health (Anon, 1996). There is no health based guideline value for silver in drinking water. In addition, warm water is not used as drinking water, even though a small amount of it may be mixed with

drinking water in taps. Mean copper concentration in Finnish apartment buildings is 80 μg/l in warm water, and 10 μg/l in cold water (Zacheus and Martikainen, 1997). The copper concentrations were low during our experiment, and the technical and aesthetical limit (1000 μg/l; Anon, 1994) was not exceeded. A health based guideline value for copper is 2000 μg/l (Anon, 1996). The control of legionellae in warm water system with the copper–silver method is possible without health risks caused by metal ions.

In our study, a small amount of copper and silver accumulated at one sampling site situated below the heat exchanger. Water tanks may contain high amounts of copper and silver when the ionisation technique is applied (Mietzner *et al*., 1997). High concentrations of silver may produce "black water", resulting from the precipitation of excess silver (States *et al*., 1998). To avoid possible excess increase in metal concentrations, it would be reasonable to periodically remove sediment from water tanks, to flush water from every point, and to follow the metal concentrations in water.

CONCLUSIONS

Even very low concentrations of metal, especially silver, ions can be effective against legionellae everywhere in the water system. Good prevention efficacy requires, however, regular use of all points in water system.

Nontuberculous mycobacteria and other heterotrophic bacteria were more tolerant to copper and silver ions than legionellae.

Properly maintained copper–silver ionisation method may be a solution to the serious problem of nosocomial legionella pneumonia.

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REFERENCES

Anon (1994) Decision of the Ministry of Social Affairs and Health relating to the quality and monitoring of water intended for human consumption. Statutes of Finland 1994, No. 74 (in Finnish).

Anon (1996) *Guidelines for drinking-water quality*, Vol. 2, 2nd ed., Health criteria and other supporting information, World Health Organization, Geneva.

Anon (1998) The control of legionellosis in hot and cold water systems. Health and Safety Executive. Supplement to The control of legionellosis including legionnaires' disease, Her Majesty's Stationary Office, London, UK.

Bailey R. K., Wyles S., Dingley M., Hesse F. and Kent G. W. (1970) The isolation of high catalase *Mycobacterium kansasii* from tap water. *Am. Rev. Respir. Dis*. **101**, 430–431.

Best M., Sattar S. A., Springthorpe V. S. and Kennedy M. E. (1988) Comparative mycobactericidal efficacy of chemical disinfectants in suspension and carrier tests. *Appl. Environ. Microbiol*. **54**(11), 2856–2858.

Bopp C. A., Sumner J. W., Morris G. K. and Wells J. G. (1981) Isolation of *Legionella* spp. from environmental water samples by low-pH treatment and use of a selective medium. *J. Clin. Microbiol*. **13**(4), 714–719.

Bullin C. H., Tanner E. I. and Collins C. H. (1970) Isolation of *Mycobacterium xenopei* from water taps. *J. Hyg. Camb*. **68**, 97–100.

Carson L. A., Petersen N. J., Favero M. S. and Aguero S. M. (1978) Growth characteristics of atypical mycobacteria in water and their comparative resistance to disinfectants. *Appl. Environ. Microbiol*. **36**(6), 839–846.

Cassells J. M., Yahya M. T., Gerba C. P. and Rose J. B. (1995) Efficacy of a combined system of copper and silver and free chlorine for inactivation of *Naegleria fowleri* amoebas in water. *Wat. Sci. Tech*. **31**(5–6), 119–122.

Colville A., Crowley J., Dearden D., Slack R. C. and Lee J. V. (1993) Outbreak of Legionnaires' disease at university hospital, Nottingham. Epidemiology, microbiology, and control. *Epidemiol. Infect*. **110**, 105–116.

Dennis P. J., Green D. and Jones B. P. C. (1984) A note on the temperature tolerance of legionella. *J. Appl. Bact*. **56**, 349–350.

Edelstein P. (1981) Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. *J. Clin. Microbiol*. **14**(3), 298–303.

Edelstein P. (1982) Comparative study of selective media for isolation of *Legionella pneumophila* from potable water. *J. Clin. Microbiol*. **16**(4), 697–699.

Edelstein P. H. (1988) Nosocomial Legionnaires' disease: a global perspective. *J. Hosp. Infect*. **11**(Suppl. A), 182–188.

Fischer-Hoch S. P., Smith M. G., Harper D. and Colbourne J. (1984) Source of *Legionella pneumophila* in a hospital hot water system. In *Legionella, Proceedings of the Second International Symposium*, eds Thornsberry *et al*., pp. 302–304. American Society for Microbiology, Washington, DC.

Iivanainen E. K., Martikainen P. J., Väänänen P. K. and Katila M.-L. (1993) Environmental factors affecting the occurrence of mycobacteria in brook waters. *Appl. Environ. Microbiol*. **59**(2), 398–404.

International Standard Organization (ISO) (1998). Water quality—Detection and enumeration of *Legionella*, ISO 11731.

Jantzen E., Tangen T. and Eng J. (1989) Gas chromatography of mycobacterial fatty acids and alcohols: diagnostic applications. *APMIS* **97**, 1037–1045.

Kauppinen J., Nousiainen T., Jantunen E., Mattila R. and Katila M.-L. (1999) Hospital water supply as a source of disseminated *Mycobacterium fortuitum* infection in a leukemia patient. *Infect. Control Hosp. Epidemiol*. **20**(5), 343–345.

Landeen L., Yahya M. and Gerba C. (1989) Efficacy of copper and silver ions and reduced levels of free chlorine in inactivation of *Legionella pneumophila*. *Appl. Environ. Microbiol*. **55**(12), 3045–3050.

Lin Y. E., Vidic R. D., Stout J. E. and Yu V. L. (1996) Individual and combined effects of copper and silver ions on inactivation of *Legionella pneumophila*. *Water Res*. **30**(8), 1905–1913.

Lin Y. E., Vidic R. D., Stout J. E. and Yu V. L. (1998a) Legionella in water distribution systems. *JAWWA* **90**(9), 112–121.

Lin Y. E., Vidic R. D., Stout J. E., McCartney C. A. and Yu V. L. (1998b) Inactivation of *Mycobacterium avium* by copper and silver ions. *Water Res.* **32**(7), 1997–2000.

Liu Z., Stout J., Tedesco L., Boldin M., Hwang C., Diven W. and Yu V. (1994) Controlled evaluation of copper-silver ionization in eradicating *Legionella pneumophila* from a hospital water distribution system. *J. Infect. Dis.* **169**, 919–922.

Liu Z., Stout J., Boldin M., Rugh J., Diven W. F. and Yu V. (1998) Intermittent use of copper–silver ionization for legionella control in water distribution systems: a potential option in buildings housing individuals at low risk of infections. *Clin. Infect. Dis.* **26**, 138–140.

Lockwood W. W., Friedman C., Bus N., Pierson C. and Gaynes R. (1989) An outbreak of *Mycobacterium terrae* in clinical specimens associated with a hospital potable water supply. *Am. Rev. Respir. Dis.* **140**, 1614–1617.

McSwiggan D. A. and Collins C. H. (1974) The isolation of *M. kansasii* and *M. xenopi* from water systems. *Tubercle* **55**, 291–297.

Merkal R. S. and Crawford J. A. (1979) Heat inactivation of *Mycobacterium avium–Mycobacterium intracellulare* complex organisms in aqueous suspension. *Appl. Environ. Microbiol.* **38**(5), 827–830.

Mietzner S., Schwille R. C., Farley A., Wald E. R., Ge J. H., States S. J., Libert T. and Wadowsky R. M. (1997) Efficacy of thermal treatment and copper–silver ionization for controlling *Legionella pneumophila* in high-volume plumbing systems in hospitals. *Am. J. Infect. Control.* **25**(6), 452–457.

Muraca P. W., Yu V. L. and Goetz A. (1990) Disinfection of water distribution systems for legionella: a review of application procedures and methodologies. *Infect. Control Hosp. Epidemiol.* **11**(2), 79–88.

Reasoner D. J. and Geldreich E. E. (1985) A new medium for the enumeration and subculture of bacteria from potable water. *Appl. Environ. Microbiol.* **49**(1), 1–7.

von Reyn C. F., Maslow J. N., Barber T. W., Falkinham J. O. III. and Arbeit R. D. (1994) Persistent colonization of potable water as a source of *Mycobacterium avium* infection in AIDS. *Lancet* **343**, 1137–1141.

Rohr U., Senger M. and Selenka F. (1996) Effect of silver and copper ions on the survival of *Legionella pneumophila* in tap water. *Zbl. Hyg.* **198**, 514–521.

Rowbotham T. J. (1984) Legionellae and amoebae. In *Legionella Proceedings of the second International Symposium*, eds C. Thornsberry, A. Balows, J. C. Feeley and W. Jakubowsky, pp. 325–327. American Society for Microbiology, Washington, DC.

Schulze-Röbbecke R. and Buchholtz K. (1992) Heat susceptibility of aquatic mycobacteria. *Appl. Environ. Microbiol.* **58**(6), 1869–1873.

Sniadick D. H., Ostroff S. M., Karlix M. A., Smithwick R. W., Schwartz B., Sprauer M. A., Silcox V. A. and Good R. C. (1993) A nosocomial pseudo-outbreak of *Mycobacterium xenopi* due to a contaminated potable water supply: lessons in prevention. *Infect. Control Hosp. Epidemiol.* **14**(11), 636–641.

States S., Kuchta J., Young W., Conley L., Ge J., Costello M., Dowling J. and Wadowsky R. (1998) Controlling legionella using copper–silver ionization. *JAWWA* **90**(9), 122–129.

Stout J. E., Lin Y. -S., Goetz A. M. and Muder R. R. (1998) Controlling legionella in hospital water systems: experience with the superheat-and-flush method and copper–silver ionization. *Infect. Control. Hosp. Epidemiol.* **19**(12), 911–914.

Thurman R. and Gerba C. (1989) The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. *CRC Crit. Rev. in Environ. Control* **18**(4), 295–315.

Tilton R. C. and Rosenberg B. (1978) Reversal of the silver inhibition of microorganisms by agar. *Appl. Environ. Microbiol.* **35**(6), 1116–1120.

Torkko P., Suutari M., Suomalainen S., Paulin L., Larsson L. and Katila M. -L. (1998) Separation among species of *Mycobacterium terrae* complex by lipid analyses: comparison with biochemical tests and 16S rRNA sequencing. *J. Clin. Microbiol.* **36**(2), 499–505.

Wadowsky R. M. and Yee R. B. (1981) Glycine-containing selective medium for isolation of *Legionellaceae* from environmental specimens. *Appl. Environ. Microbiol.* **42**(5), 768–772.

Wallace Jr. R. J., Brown B. A. and Griffith D. E. (1998) Nosocomial outbreaks/pseudo-outbreaks caused by nontuberculous mycobacteria. *Annu. Rev. Microbiol.* **52**, 453–490.

Zacheus O. M. and Martikainen P. J. (1994) Occurrence of legionellae in hot water distribution systems of Finnish apartment buildings. *Can. J. Microbiol.* **40**, 993–999.

Zacheus O. M. and Martikainen P. J. (1997) Physicochemical quality of drinking and hot waters in Finnish buildings originated from groundwater or surface water plants. *Sci. Total Environ.* **204**, 1–10.