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# The control of Legionella pneumophila and other biofilm-related pathogens in the tapwater at Tjongerschans hospital.

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***In the period 2017 to mid-2020, Tjongerschans hospital in Heerenveen suffered from a Legionella infection in the water supply system. Thermal/physical management of the tap water did not result in a permanent decrease in Legionella concentrations. The use of copper and silver ionisation, with Holland Water's HW Bifipro® system, did ensure effective control of the excessively high Legionella pneumophila concentrations in drinking water. Within 6 months the tap water was Legionella-free. As a side effect, the pathogens Pseudomonas aeruginosa and Aeromonas spp. were also effectively addressed.***

Tap water in complex drinking water installations - such as in hospitals - is vulnerable to the outbreak of Legionella pneumophila (*L. pneumophila*) and other pathogens, including Escherichia coli (*E. coli*), Pseudomonas aeruginosa (*P. aeruginosa*) and Aeromonas spp. Complex drinking water installations often contain dead pipe parts, or pipe parts with stagnant water. Here the conditions - stagnant water, higher temperatures, and biofilm formation - are ideal for the growth of these bacteria. This was also the case at Tjongerschans hospital. From 2019 to mid-2020, *L. pneumophila* concentrations were regulated > 15,000 CFU/L, including those of the extremely dangerous Legionella variant serotype 1.

## **Copper and silver ionisation**

The first step in tackling Legionella in the Netherlands is thermal/physical management. If that does not work, alternative management techniques, such as copper and silver ionisation, may be used. The fact that thermal/physical management does not always work is evident from the fact that approximately 1,000 copper and silver ionisation systems are operational in the Netherlands. Holland Water offers both copper and silver ionisation systems for the treatment of drinking water - the HW Bifipro® system - and for cooling tower water - the HW Bifipro® Cool system. Not only is Legionella effectively combated, but the use of chemicals can also be eliminated with all the (environmental) benefits that this entails. If the building is legionella-free for 1 year, the hot water temperature may also be reduced by 10 °C (return in boiler 50 °C). This results in significant energy savings and a reduction in CO2 emissions.

The effect of copper and silver ionisation has been substantiated in dozens of scientific articles (e.g., Liu et al., 1994, 1998; Lin et al., 1996; Kiwa, 2006; Rohr et al., 1999; Lin et al., 2011; RIVM, 2012; Walraven et al., 2016; Cloutman-Green et al., 2019). According to the literature, copper and silver ionisation is the only disinfection technique for Legionella that has been validated according to the 4-step evaluation criteria: demonstrated, reported, reviewed, and confirmed in vitro and in vivo effectiveness (Lin et al., 2011). In addition, according to the Dutch Board for the Authorisation of Plant Protection Products and Biocides (CTGB), there are no human risks for the use of copper and silver ionisation (CTGB, 2007).

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### **Also effective against other potential pathogens**

Copper/silver ionisation is not only effective against Legionella, but also against various other bacteria, viruses, amoebae, and protozoa with cilia (Walraven and Chapman, 2016). This has been demonstrated by several scientific in vitro studies and a single in vivo study (in a model water supply system). However, the number of practical studies is limited. Because there has also been colonisation in Tjongerschans hospital with an Aeromonas spp. (100 – 1,100 CFU/100 ml), this was an excellent opportunity to investigate whether Holland Water's HW Bifipro® system is also effective in combating Aeromonas spp. In addition, E. coli and P. aeruginosa were also included in the study. E. coli is a microbiological parameter according to the Drinking Water Decree with a limit value of 0 CFU/100 ml. P. aeruginosa is considered an opportunistic pathogen in Dutch drinking water that should be investigated more (e.g., KWR, 2018). Diseases caused by these other (potential) pathogens are not obliged to be reported but it is estimated that they cause even more cases than disease-causing Legionella non-pneumophila species (Scheffer, 2021). Under 'Pathogens Studied', the human risks of the investigated pathogens are briefly described.

## **Pathogens studied**

### **Legionella pneumophila**

This bacterium can cause legionellosis, also known as Legionnaires' disease. This is a severe form of pneumonia. The disease can occur when water vapour is inhaled with these bacteria, for example during showering or through mist from whirlpools, spray systems and wet cooling towers.

### **Escherichia coli**

This is an intestinal bacterium that occurs in humans and warm-blooded animals. This bacterium helps in the digestion of food. Although these bacteria have a positive function in the digestion of food, it can pose a danger in wrong places in the body. If certain types of E. coli bacteria are present in drinking water, this can result in nausea, abdominal cramps, vomiting and diarrhoea when ingested, among other things. In addition, E. coli is the most common causative agent of urinary tract infections.

### **Aeromonas spp**

This is a group of bacteria that is common in fresh and brackish surface waters. However, it also occurs in drinking water, possibly because drinking water is prepared from surface water that is contaminated with this bacterium. Important pathogens in the Aeromonas group are A. hydrophila, A. caviae and A. veronii biovar sobria. Diseases related to these bacteria are gastroenteritis (inflammation of the stomach and intestines) and wound infections.

### **Pseudomonas aeruginosa**

This bacterium is regarded as one of the hospital bacteria, which are responsible for many human infections, including wound, urinary tract and ear infections. Meningitis has also been observed occasionally. Because this bacterium has become resistant to the commonly used types of antibiotics, combating infection with P. aeruginosa is difficult.

### **Investigation at Tjongerschans hospital**

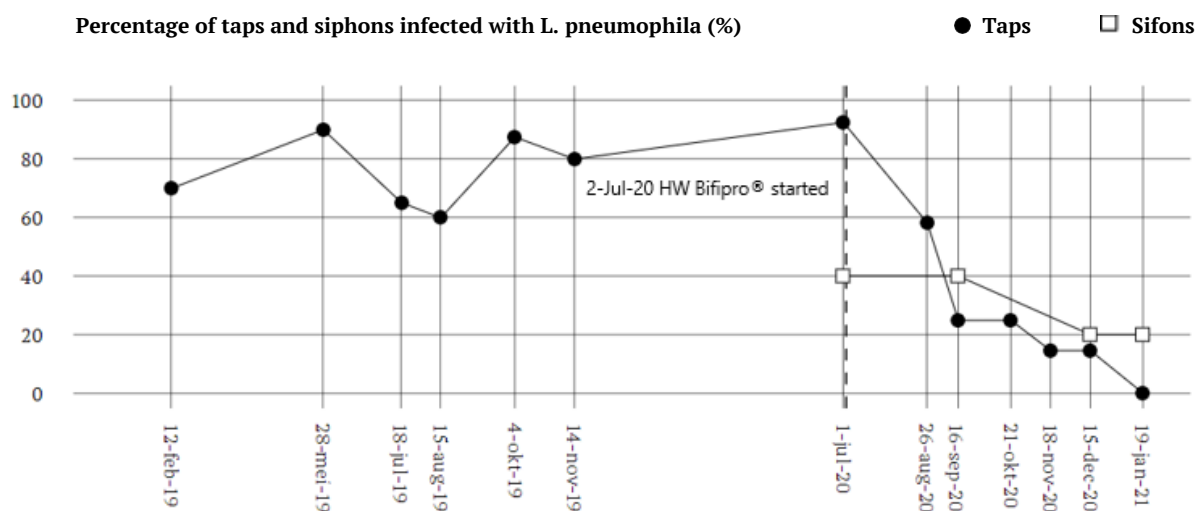
On 1 July 2020, the 0-study was carried out (for switching on HW Bifipro® system). 12 taps were sampled and analysed for L. pneumophila, E. coli, P. aeruginosa, Aeromonas spp., colony count and copper and silver concentrations. In addition, 5 washbasin siphons were also sampled and analysed for the same parameters.

After installation, the HW Bifipro® system was switched on July 2, 2020. The same taps and siphons were monitored monthly and 2-monthly respectively after switching on the HW Bifipro® system. The L. pneumophila concentrations, colony counts and copper and silver concentrations in the taps were sampled and analysed monthly and the E. coli, P. aeruginosa and Aeromonas spp. concentrations 2-monthly.

The total monitoring duration was 6 months. The siphons were included in this study because they are considered as potential sources of infection. The sampling was carried out by C-mark in accordance with NEN-EN-ISO 19458. The analyses were carried out by Eurofins. Table 1 shows the analytical methods and the monitoring frequency per installation component and parameters.

Figure 1 shows the percentage of taps and siphons infected with *L. pneumophila* (> 100 CFU/L) plotted against time (from early February 2019 to January 2021). The measurement results for 1 July 2020 were obtained by Tjongerschans hospital during regular Legionella investigation. Tables 2 and 3 summarise the analysis results of tap water from the taps and siphons.

**Figure 1.** Percentage of taps and siphons infected with *L. pneumophila* versus the monitoring rounds



### Legionella in the taps

Before switching on the system, approximately 60 to 90 % of the taps examined were contaminated with *L. pneumophila* (Figure 1). The *L. pneumophila* concentrations ranged between < 100 and > 15,000 CFU/l (Table 2). Within 6 months of switching on the HW Bifipro® system, the infection rate in the taps gradually decreased to 0% infections (Figure 1). After 3 months, the decline levelled off. This arose because 1 tap could not be flushed because this tap was located in a department with COVID-19 patients, which was therefore inaccessible. Copper and silver concentrations in the pipes varied between < 2 and 1,500 µg Cu/l during copper and silver ionisation and between < 3 and 66 µg Ag/l with an average of 730 µg Cu/l and 38 µg Ag/l (Table 2). Part of the copper comes from the copper pipes, namely < 2 to 1,000 µg Cu/l with an average of 472 µg/l (Table 2: 0 situation). The dosed copper and silver concentrations meet the requirements of the CTGB and the Dutch Drinking Water Decree.

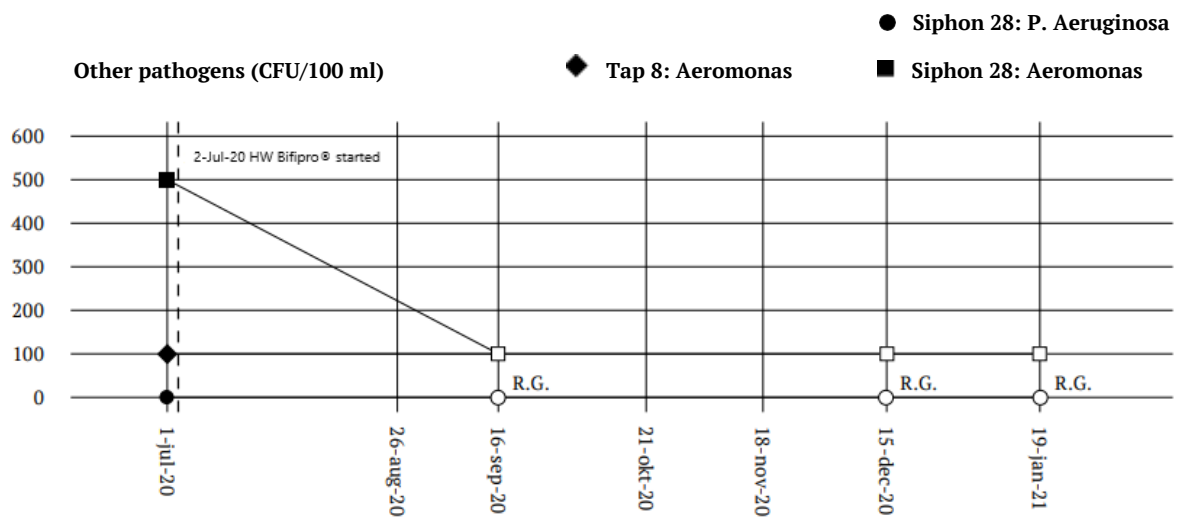
### Legionella in the siphons

The Legionella infection rate of the siphons was 40 % in the 0 situation (Figure 2), with concentrations ranging between < 100 and 1,700 CFU/l (Table 3). In the siphons, the infection rate fell to 20 %: 1 siphon was still infected after 6 months (Table 2: 500 CFU/l). This siphon is located in the same room where the increased *L. pneumophila* concentration in the tap was measured. This area was not accessible for flushing due to a COVID-19 infection. The colony count in this siphon, as well as in 2 other siphons, is still high after 6 months, namely > 3000 CFU/ml (Table 3). This confirms the hypothesis that siphons may be hotbeds of pathogens. Copper and silver concentrations in the siphons varied between 110 and 2,000 µg Cu/l and between 17 and 62 µg Ag/l during copper and silver ionisation with an average of 771 µg Cu/l and 48 µg Ag/l (Table 3). Part of the copper comes from the copper pipes, namely 420 to 900 µg Cu/l with an average of 608 µg/l (Table 3: 0 situation). The dosed copper and silver concentrations meet the requirements of the CTGB and the Dutch Drinking Water Decree.

### Other pathogens in the taps and siphons

In the 0 study, elevated *P. aeruginosa* and *Aeromonas* spp. concentrations were measured at 3 sampling points (Tables 2 and 3). These are shown in Figure 2. After 2 months, *P. aeruginosa* concentrations in these sampling points were 0 CFU/100 ml and *Aeromonas* spp. concentrations < 100 CFU/100 ml. In all other sampling points, *E. coli* and *P. aeruginosa* concentrations were 0 CFU/100 ml and *Aeromonas* spp. concentrations were < 100 CFU/100 ml (Tables 2 and 3). Although the number of infections with these bacteria was limited, this research shows that copper and silver ionisation with the HW Bifipro® system is effective in combating them. More practical research into the effectiveness for these pathogens, and possibly other relevant bacteria, is desirable.

**Figure 2.** The *P. aeruginosa* and *Aeromonas* concentrations in siphon 28 and tap 8. The open symbols refer to measured values of 0 or < reporting limit (R.G.).



### HW Bifipro® System Effective

This research shows that copper and silver ionisation with the Bifipro® system is highly effective in the control of *L. pneumophila* and other investigated pathogens. Within 2 months the infections with *P. aeruginosa* and *Aeromonas* spp. were under control and within 6 months the persistent *Legionella* infection in Tjongerschans hospital was effectively controlled. Although the flushing frequency may be increased when the HW Bifipro® system is deployed and the location is legionella-free, it is recommended that all taps be flushed at least every two weeks. It is important that this also happens when rooms (e.g., due to Covid-19) are taken out of use. Extra attention should be paid to the siphons. The siphons are not yet a mandatory part of the legionella management plan.

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**Table 1.** The monitoring frequency per installation component and parameter, and the analytical methods used.

Parameter	Monitoring frequency		Analysis
	Tap points	Siphons	
<i>L. pneumophila</i>	monthly	2-monthly	NEN-EN-ISO 11731
<i>E. coli</i>	2-monthly	2-monthly	NEN-EN-ISO 93081
<i>P. Aeruginosa</i>	2-monthly	2-monthly	NEN-EN-ISO 16266
<i>Aeromonas</i>	2-monthly	2-monthly	NEN-EN-ISO 6223
Colony count	monthly	2-monthly	NEN-EN-ISO 6222
Copper	monthly	2-monthly	NEN-EN-ISO 17294-2
Silver	monthly	2-monthly	NEN-EN-ISO 17294

**Table 2.** The concentrations of the investigated parameters in the taps: before and after the start of copper and silver ionisation with the HW Bifipro® system.

Parameter	Before starting copper-silver ionisation		After starting copper-silver ionisation					
	Measure values	Remark	Round 1 26/8/20	Round 2 16/9/20	Round 3 21/10/20	Round 4 18/11/20	Round 5 15/12/20	Round 6 19/1/21
Legionella pneumophila (CFU/l)	<100 to >15.000	<100 to 1.400	<100 to 1.400	<100 to 1.800	<100 to 300	<100 to 200	<100 to 15.000 #1	<100
E. coli (CFU/100 ml)	0	0	0	0	0	0	0	0
P. Aeruginosa (CFU/100 ml)	0	0	0	0	0	0	0	0
Aeromonas (CFU/100 ml)	<100 to 100	<100	<100	<100	<100	<100	<100	<100
Colony count (CFU/ml)	<1 to 80	30 to 170	30 to 170	<1 to 260	30 to 100	3 to 120	6 to 170	3 to 80
Copper (µg/l)	<2 to 1.000	300 to 1.000	300 to 1.000	<2 to 1.200	320 to 1.300	110 to 1.300	300 to 1.300	310 to 1.500
Silver (µg/l)	<3	19 to 52	19 to 52	<3 to 58	14 to 47	6.7 to 45	17 to 66	11 to 66

#1 This tap was not flushed because there was a COVID-19 infection in this room.

**Table 3.** The concentrations of the investigated parameters in the siphons: before and after the start of copper and silver ionisation with the HW Bifipro® system.

Parameter	Before starting copper-silver ionisation		After starting copper-silver ionisation		
	Measure values	Remark	Round 2 16/9/20	Round 5 15/12/20	Round 6 19/1/21
Legionella pneumophila (CFU/l)	< 100 to > 1.700	0 situation	< 100 to 4,500	< 100 to 600 #1	< 100 to 500 #1
E. coli (CFU/100 ml)	0	0 situation	0	0	0
P. Aeruginosa (CFU/100 ml)	0 to 4	0 situation	0	0	0
Aeromonas (CFU/100 ml)	< 100 to 500	0 situation	< 100	< 100	< 100
Colony count (CFU/ml)	870 to > 3.000	0 situation	390 to > 3,000	110 to > 3,000	180 to > 3,000
Copper (µg/l)	420 to 900	0 situation	590 to 2,000	510 to 930	430 to 920
Silver (µg/l)	< 3	0 situation	17 to 49	41 to 61	53 to 62

#1 This siphon was not flushed because there was a COVID-19 infection in this room.