Outbreak investigation and epidemiology - from practice to science - .
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Citation for published version (APA):
Cold tap water as a source of fatal nosocomial pneumonia caused by Legionella pneumophila in a rehabilitation centre

6.1 ABSTRACT

This chapter discusses the technical, microbiological and epidemiological investigation following 2 cases of fatal Legionella pneumonia. Faced with two nosocomial cases in a rehabilitation centre in the South of Limburg, the Netherlands, the water supply was investigated. Water temperatures from different taps were measured. Legionella cultures were made from respiratory patients’ specimens, water samples and smears from all mixing taps (used in showers), samples from hot and cold water taps from the infected ward and from the five other wards. The strains were typed by serotyping and polymerase chain reaction. The circulating cold water sometimes warmed up to 40°C (within the Legionella growth range). From the sputum of the two male patients with rheumatoid arthritis who died of Legionella pneumonia the same Legionella pneumophila (serotype 1) was cultured as from the water supply. Of the showers on the contaminated ward 19% (12/63) were positive for Legionella as were 59% (35/59) of the cold water taps. Cultures from the hot water supply were negative just like control cultures from five other wards and swabs from showerheads and hoses. The cold water tubes ran next to the hot water tubes and the central heating system in the same shaft. On the infected ward patients were absent during the weekends. As one of the subsequent measures, the cold water pipes were relocated to another shaft.

The combination of an elevated cold water temperature caused by heating along a distance by nearby hot water and heating piping and the regular stasis of water during the weekends when the ward was closed, most probably stimulated the multiplication of Legionella in the water supply. In order to minimize contamination of cold water, its temperature must be below 20°C. Surveillance of intramural water systems is necessary to prevent nosocomial infections.
6.2 INTRODUCTION

After two patients developed a fatal *Legionella* pneumonia (Legionnaries’ disease) in a rehabilitation centre, we performed a technical, microbiological and epidemiological investigation which is discussed in this chapter.

*Legionella pneumophila* is the bacterium responsible for this severe pneumonia.14 A *Legionella* pneumonia develops in 2–10 days after infection. It is assumed that after exposure older persons with pre-existing morbidity are at greater risk of Legionnaires’ disease than healthy individuals. In addition, mortality among patients who acquire a nosocomial infection is higher (40%) than among those infected outside an institution (20%). In 1997, the average mortality among patients in 24 European countries was 10% (136/1360), according to the surveillance system of the European Working Group for Legionella Infections.

In the Netherlands, *Legionella* pneumonia is officially reported on average 45 times per year. In three-quarters of the cases, the source is known and one-third of these infections originates from the Netherlands. One-third of the Dutch *Legionella* pneumonia cases (8% of 45 cases) appear to be related to a health-care facility. As a result of the investigation described in the present paper, the IGZ, in a letter dated July 1997, called for measures to be taken within the various institutions to prevent intramural *Legionella* infections and to include *Legionella* in the diagnostic work-up of any pneumonia cases of unknown cause. Most likely, this appeal has contributed to the five-fold increase in 1997 of *Legionella* notifications connected with health-care facilities (10 cases), compared to an annual average of 2 intramural cases over the previous four years. In Europe as a whole 16% (218/1360) of the *Legionella* cases are thought to be related to medical institutions.5

6.3 HISTORY

The first inspection of the water supply system of the rehabilitation centre took place after a 58-year-old man (patient A), admitted to a four-year-old ward on the top floor, suffered a fatal nosocomial *Legionella* pneumonia at the end of 1996. The contaminated ward was used to nurse patients with rheumatoid arthritis or chronic pain during week-days. Every room had a sink and a shower with hot and cold water. A separate room was used to clean the equipment such as medical instruments, aids and appliances and other materials. The entire copper-piped water supply system, especially the hot water segment leaving the boiler and the section from the thermostat to the outlet, was carefully examined, both on the building plan and in practice, for weak spots and dead pipe-legs. The assessment showed no technical defects. The temperature of the boiler was checked and found to be above 70°C. No *Legionella* could be cultured from the water of the shower in the patient’s room.

A shower in an adapted bathroom with special facilities on the same ward gave the
only positive test result, with 87,000 colony forming units per litre (CFU/l). The *Legionella* strain found in water samples from this shower had the same serotype (serotype 1) as the bacteria found in respiratory specimens of the patient (figure 6.1). The central water supply from the waterworks tested negative for *Legionella* in the microbiological analysis.

**Preventive measures**

A plan of action for prevention and surveillance was drafted in order to stop additional nosocomial infections. We assumed that the *Legionella* bacteria would survive and multiply during the weekends in the polyethylene hoses of the showers. Therefore, all showers were thermally disinfected after the weekends by flushing them for ten minutes with water at a temperature of at least 70°C. All hoses in the showers were replaced because of the possible formation of a biofilm. On a monthly basis, water samples from the mixing taps in the showers on the ward were tested for *Legionella* and the water company checked their water supply for a year. During this year, no cases of *Legionella* pneumonia were seen, neither did any of the hot water samples test positive.

The effect of these precautions did not last, however. In 1997 - one year after the first patient had been diagnosed - *Legionella* was again cultured from a hot water sample, although this specimen had been taken from a room not open to the patients. Shortly after this, a new fatal case of *Legionella* pneumonia occurred in a 48-year-old man (patient B).

6.4 METHODS

In view of the positive water sample and the second fatal *Legionella* pneumonia technical inspections, water temperature measurements and microbiological analyses with cultures and serotyping were once again performed. Unlike the first time, these investigations now included the cold water supply.

**Technical inspection and water temperature measurement**

The rehabilitation centre and the water company checked the entire water supply system. The temperature of running water at the various hot and cold water taps in the contaminated wards and in control wards was recorded for a period of 10 minutes at maximum flow with a fast digital thermometer (measurement accuracy 0.1°C).

**Microbiological analysis**

Every week, water samples were taken from the showers and the taps in the 14 rooms on the contaminated wards, and a similar number of samples was collected from control rooms in the five other units. Smears of the showerheads and hoses were made. After the first two measurements of water contamination, water samples were also collected at two
different moments in time; before and five minutes after opening the taps. This procedure was needed to answer the question whether Legionella might be present in the shower hoses or the distal part of the water pipes only, or whether the whole water supply system was contaminated. Every water sample was labelled with the room number, the exact point where the sample had been taken and the day and time of collection, in order to correctly identify the results later. These specimens were sent for culturing on the same day.

**Culture method**

The laboratory cultured the sediment of a sample of 1 litre water, passed through a bacteriological micropore filter (pore diameter 20 μm). This filter was then transferred to a 60 ml bottle filled with approximately 40 glass beads and 2.5 ml of a phosphate buffered normal saline solution (PBS) with a pH of 7.2. After that, the filter was broken and mixed with the solution for 15 seconds. This suspension was divided into two fractions. The first portion was placed in a water bath at a temperature of 50°C for 30 minutes (the so-called “treated” specimen), while the second portion was left untreated. Subsequently a 1:10 dilution in PBS was made of part of both specimens. Of the four samples remaining (diluted, undiluted, treated and untreated) 0.1 ml was added to three different culture media (basic buffered charcoal yeast extract (BCYE) agar, BCYE agar with antibiotics and BCYE agar with cysteine). After a standard culture procedure of one week, the number of CFU/l was determined by multiplying the total number of colonies present with the dilution factor 25. For this reason, the quantity of Legionella found was always a multiple of the minimal count of 25 CFU/l.

**Legionella typing**

The potential link between the isolates of the two patients involved and the water samples was examined using of serotyping and the polymerase chain reaction (PCR). To determine the DNA profile, a so-called “touch-down” PCR was performed with an annealing temperature increasing to 61°C, using the DNA primers ERIC-I, ERIC-II, REP-I and REP-II. The PCR products were analysed by means of electrophoresis in 1% agarose for two hours at 100 V.

### 6.5 RESULTS

**Technical inspection and water temperature measurement**

Just as in 1996, technical investigations revealed no defects, but this time it was noticed that the cold water pipes leading from the ground floor to the top floor were in the same shaft with and closely fitted to the hot water pipes and the heating tubes. The temperature in this shaft could rise to a maximum of 40°C. During the weekends, there was no flow of water through these pipes because the patients of the wards then went on weekend leave.
The temperature of the water from the hot water taps exceeded 70°C within 30 seconds. The temperature of the water from the cold water taps turned out to rise up to 42°C at some moment in time, which is well within the range of 20-50°C required for Legionella to multiply. (Although Legionella can live in water of between 50 and 55°C without growing, it will not survive temperatures above 55°C.) Figure 6.2 shows the temperature curve of the flow of cold water, which had been stagnant for 24 hours, in the contaminated ward on the top floor after opening the taps at the beginning of the corridor (closest to the shaft) and at the end, respectively. This figure illustrates that a volume of warm water flows from the cold water pipes in the central shaft at some point in time, depending on the position of the tap relative to the shaft. After the process of culturing, 19% (12/63) of the mixing taps in the contaminated ward tested positive for Legionella (median 175 CFU/l, range 25-20,000 CFU/l), versus 59% (35/59) of the cold water taps (median 175 CFU/l, range 25-2,000 CFU/l). The cultures from the hot water taps (4) were found to be negative for Legionella, like the control cultures from five other departments (46) as well as the smears of the showerheads and hoses (16).

**FIGURE 6.1.** Genotyping pattern (electrophoresis image) obtained after polymerase chain reaction on isolates of Legionella pneumophila serogroup I of two patients (Heerlen A (from bronchoalveolar lavage fluid) and Heerlen B (from bronchial secretion) in lane 3 and lane 5, respectively, and from water samples of the patients’ rooms (room A and room B in lane 2 and lane 4, respectively). Lane 1 and lane 13 represent a control strain from the National Institute for Public Health and the Environment (RIVM). Lanes 6-12 show unrelated strains of L. pneumophila (serogroup I) found in 1997, in order of place of origin.
Microbiological analysis

Samples were taken at 23 different moments in time, from 0 seconds to 5 minutes after opening the taps. It was assumed that most legionellae would be found at 0 seconds, but the variation was large. In 17% of the double measurements (4/23), the first sample produced most colonies, while the final sample, after 5 minutes, did so in 15% of the measurements (3/23).

Figure 6.2. Temperature curve of the Legionella pneumophila contaminated water supply system in the corridor of a ward of a rehabilitation centre, upon complete opening of two cold water taps after the water in the system has been stagnant for 24 hours: (—) tap at the beginning of the hallway close to the vertical shaft also containing the hot water pipes and heating tubes; (—■—) tap at the end of the hallway.

In 26% of the measurements (6/23), the first specimen was negative, while the sample after 5 minutes proved positive, but the opposite result was found in 30% of the measurements (7/23). The other three tests were both negative. In addition, a large variance was discovered between the weekly measurements of the same tap.

Legionella typing

Serotyping showed that the cultured L. pneumophila strain belonged to serogroup 1. According to the PCR results, the DNA profiles of the Legionella isolates from the patients were identical with the strains found in the cold water supply of their ward (figure 6.2).
Figure 6.3. Overview of the percentages of positive and negative cultures for Legionella pneumophila from water samples of mixing taps and cold water taps before and after preventive measurements in control Legionella infection in a ward of a rehabilitation centre (figures in the bars represent absolute numbers): (•) negative culture; (■) positive culture: number of legionellae <100 colony forming units per litre (CFU/l); (□) positive culture: number of legionellae ≥100 CFU/l.

6.6 INTERVENTIONS

For the short term, further infections were prevented by means of thermal and chemical decontamination (chlorination). The construction of a ring-line and the installation of a pump in the cold water supply system ensured a continuous flow of water. Weekly water samples in the first half-year and monthly follow-up in the second half year showed a significant reduction of Legionella presence. Figure 6.3 demonstrates that even after the interventions, 7% (2/27) of all mixing tap samples and 15% (9/62) of the cold water supply samples still tested positive for Legionella. During the follow-up assessment, all culture results were 25 CFU/l, apart from one sample containing 300 CFU/l. This sample had been taken from a scarcely used cold water tap at the end of the hallway, which could in fact be regarded as a dead pipe leg. After the positive test result, this tap was removed. By way of definitive solution, the cold water pipes were recently relocated to another, existing shaft without hot water pipes inside.
6.7 DISCUSSION

It is most likely that both patients were infected through the water from their shower, which was contaminated in the cold water pipes. It was verified that the cold water supply system was the source of the two fatal cases of *Legionella*. A combination of events probably allowed *Legionella* to multiply in the water inside the cold water pipes in the rehabilitation centre. On the one hand, as a consequence of the nearness of the hot water pipes and the heating tubes, the ambient temperature in the shaft caused spontaneous warming of the cold water, resulting in an elevated cold water temperature. On the other hand, this process of heating up could take place over a considerable length of time at regular intervals, because of stasis of the water during the weekends, when the ward was closed.

Therefore, preventing nosocomial *Legionella* pneumonia requires not only the temperature of the hot water supply to be checked but also critical attention to be given to the temperature of the cold water system.

Technical inspection and water temperature measurements are simple procedures. The microbiological analysis of the water supply system, however, presents a number of questions: how many water samples should be taken, at what frequency and from which taps, what risk can be deduced from the quantity of *Legionella* found and which interventions should be taken at what point(s) in time?

In this survey, 19% of the water samples from the mixing taps and 59% of the water samples from the cold water taps tested positive for *Legionella* during a 6-months follow-up screening, but preventive measures reduced these figures to 7% and 15%, respectively. In two similar contaminated hospital wards described in a Canadian study, one year of chemical decontamination (chlorination) resulted in 12% (51/410) and 11% (46/410) of the water samples testing positive for *L. pneumophila*, against 42% (284/680) and 23% (156/680) during a three-year period without any intervention. In the first unit, 24 patients developed nosocomial *Legionella* pneumonia, against 4 patients in the second department. In a hospital for ear, nose, and throat oncology, *Legionella* was responsible for 30% of all cases of acquired pneumonia, at a time when 67% of the water taps tested positive for *Legionella*. After preventive measures reduced this number to 4%, no further patients were diagnosed with *Legionella* pneumonia. Only a fraction of the water samples from a contaminated water supply system is evidently found to be positive for *Legionella*. The data suggest that when more than one-third of the water taps are *Legionella* positive, there is a considerable risk of nosocomial *Legionella* infections.

**Percentage of positive water taps**

For the purpose of analysis, the percentage of positive water taps seems to be a more reliable figure than the number of bacteria cultured from each individual tap. It has become evident that a single tap can test negative for *Legionella* at a specific point in time but turn
out to test positive one week later. In addition, substantial differences can be found between
the concentrations of *Legionella* in water samples taken before opening the taps and those
obtained five minutes afterwards, indicating that this practice of double sampling is not
meaningful. Furthermore, the numbers of bacteria cultured from the various taps were not
very consistent. Nevertheless, a relationship between the quantities of legionellea found and
the incidence of nosocomial legionellosis seems apparent. The number of legionellae in the
cold water samples in our survey varied between 25 and 20,000 CFU/l; 40% of all positive
samples contained more than 100 CFU/l. Total eradication of *Legionella* from the water
supply system appeared impossible, as a small percentage of the regular control samples
remained positive in spite of the interventions. With the exception of one sample mentioned
above, however, a level of contamination above 100 CFU/l was no longer seen.

**Risk of infection**

Freije and Barnaree point out that at a level of contamination less than 1,000 CFU/l
low-risk persons are not expected to be at risk of Legionnaires' disease. A level of contamination
between 1,000 and 10,000 CFU/l is believed to cause a risk of infection ranging between low
and increased, suggesting the need for disinfection of the water supply system. Above 10,000
CFU/l, the risk of an outbreak of legionellosis is regarded as high or even very high, and
decontamination is indicated. A lower threshold should be defined for individuals with a
high risk of developing *Legionella* pneumonia, and as a result of our study we recommend
100 CFU/l. Preventive measures against legionellosis, such as thermal and chemical
(chlorinating) decontamination, only seem to have a temporary effect. The distribution of
sterile water to patients for the purpose of drinking, brushing their teeth and taking their
medication, as practised occasionally, is unlikely to be effective since the source of infection
is not eliminated. Only technical modifications to the water supply system seem to offer an
adequate solution. Installation of an automatic temperature gauge, yielding continuous
measurements of the water temperature, could be useful if it is connected to both the cold
and hot water system, giving a warning signal when the water temperature rises above 20°C
or drops below 60°C respectively.

**6.8 CONCLUSION**

Despite previous warnings against the risk of spontaneous warming of cold water as
a result of nearby hot water pipes or heating tubes, limited attention is still given to the risk
of *Legionella* in the cold water supply. To our knowledge, this report is the first to
demonstrate the clinical relevance of *Legionella* in the cold water supply of a health care
institution, causing nosocomial infections. These findings are especially relevant in
situations when wards are not in permanent use (7 days a week). Apart from technical
inspection and water temperature measurements, microbiological surveillance of the water supply systems in intramural health care facilities appears to be an effective method for preventing nosocomial legionellosis. If more than one-third of all water taps are contaminated with *Legionella*, further investigations and interventions are indicated. In the case of individuals at high risk of Legionnaires’ disease, like many inpatients, concentrations below 100 CFU/l could be considered safe.

6.9 REFERENCES

6.10 EPICRISE: CONTROLLING LEGIONELLA IN DRINKING WATER SYSTEMS

*Legionella* is increasingly recognised as a significant cause of pneumonia, both nosocomial and community-acquired. Outbreaks crop up regularly because improvements in diagnostic tests have facilitated recognition of the infection. Legionnaires’ disease cannot be diagnosed on the basis of clinical presentation or chest radiograph alone; specific tests are necessary. Many cases are missed because these tests are not routinely used on pneumonia patients in most countries. In the United States, it has been estimated that less than 5% of cases are reported to public health authorities.

Many *Legionella* infections originate from drinking-water systems, the most difficult of water systems in the control of *Legionella* contamination. Such contamination of drinking-water systems in health-care facilities is common. In a national survey of US hospitals, 34% reported recovery of the bacteria from their plumbing system and 29% reported nosocomial Legionnaires’ disease. *Legionella* has been recovered from up to 100% of hospitals in a single area. Because of this ubiquity, the question is not whether *Legionella* bacteria are present, but rather if circumstances (i.e. temperature, stagnation, biofilm) favour amplification. Policies for prevention of legionellosis would be simple if a ‘cut-off’ level of bacterial concentrations existed above which the risk of *Legionella* infection became unacceptable. However, to this day the predictive value of any *Legionella* quantification in water samples remains unclear. *Legionella* concentrations fluctuate considerably over time and there is significant inter-laboratory sensitivity variation. Nevertheless, common sense dictates that finding *Legionella* in a high proportion of fixtures or finding very high *Legionella* counts in any water sample are signs of danger.

All methods for preventing the growth of *Legionella* in drinking water have drawbacks and none is 100% efficacious. There remains a lot of controversy about optimal control strategies. Microbiological testing and decontamination efforts can be expensive, so their implementation is not justified unless they can reduce the risk of disease by a reasonable degree. Decontamination efforts should be sustained, systematic, and not prone to human error. Any control effort should include an assessment of technical weaknesses of the water system. *Legionella* growth is most likely in areas where water stagnates. Moreover, heat and disinfectants such as chlorine do not penetrate these areas well. The prerequisite for success of any *Legionella*-prevention measure is thus removal of ‘dead legs’, parts of the water system that are not used for extended periods of time, and other structural factors that may cause stasis.

Temporarily increasing water temperature (by superheat-and-flush) will reduce *Legionella* counts for a short while. For long-term effect, the water temperature should be maintained above 50° C continuously in every part of the hot-water system. A disadvantage of this method is the risk of scalding. In addition, increasing the hot-water temperature may warm up the cold-water because of heat exchange between the two systems. The result could
be an increase in cold-water-associated *Legionella* transmission.\(^5\) Cold-water temperatures can also increase by ambient heating when outside temperatures are high. In buildings that are used intermittently, such as day-care clinics, this effect may become important because of prolonged periods of stasis in the cold-water system.\(^6\)

Free chlorine and chlorine dioxide are effective if their concentration is adequate. However, high chlorine concentrations may corrode plumbing materials, and municipal water plants commonly do not use sufficient concentrations for *Legionella* control. Moreover, free chlorine and chlorine dioxide penetrate not very well into the biofilm where *Legionella* lives,\(^7\) nor do they reach peripheral areas of the plumbing system very well. Monochloramine may be considerably more effective than free chlorine in municipal water plants or in individual hospitals.\(^8\) For drinking-water systems, residual disinfection (maintenance of disinfectant throughout the system) with monochloramine may become accepted as an additional and potentially very cost-effective method for prevention of nosocomial and community-acquired Legionnaires’ disease.\(^9\)

Copper-silver ionisation for control of *Legionella* has given variable results. It has reduced *Legionella* counts when used with continuous chlorine injection.\(^9\) Unfortunately many subsequent studies on the effectiveness of copper-silver for *Legionella* control have not reported chlorine concentrations, so it remains unclear how much of the effect is attributable to copper-silver and how-much to chlorine. In several hospitals, *Legionella* have been recovered from water systems and cases of Legionnaires’ disease have occurred despite copper-silver ionisation.\(^1\) Tolerance of *Legionella* to long-term exposure to silver has been reported.\(^2\) Ozone and ultraviolet light also seem to reduce growth of *Legionella*. However, the effect is local, short-lived, and does penetrate the biofilm poorly.

The mainstay of *Legionella*-control in drinking-water systems is thus the checking of water temperatures and disinfectant concentrations at the point of use, together with the prevention of stasis of water. Any hospital or other institution housing with susceptible persons should make a serious effort to identify and remove all dead legs in the water system and to make other necessary adjustments. These measures should be undertaken irrespective of whether nosocomial transmission has been identified or whether *Legionella* have been recovered from water. Microbiological testing of water (e.g. twice a year) could then be considered, as a back-up method, to identify possible lapses in control measures. It is also important that physicians are aware that *Legionella* is a common cause of pneumonia and that they should consider requesting *Legionella* diagnostic tests on severe pneumonia patients to ensure adequate treatment and timely recognition of outbreaks.
6.11 REFERENCES EPICRISE

MRSA-bacterie teistert Kerkraadse Huisverpleging

Zeventien mensen besmet geraakt

De Hamboskliniek in Hamboskerk in Kerkrade nam maatregelen om verdere besmetting bij de Kerkraadse Huisverpleging te voorkomen. De directie van de Hamboskliniek in Kerkrade meldt dat de MRSA-bacterie, een van de gevaarlijkste bacteriën, in de verpleeghuizen verwekt is.

De besmetting werd herkend bij een woningwachter die werkzaam is in het verpleeghuis. Volgens de directie van de Hamboskliniek is de besmetting aan het licht gekomen door een interne onderzoek in het laboratorium van de kliniek. Bij de onderzoek is onder andere een bacterie geïdentificeerd die MRSA genoemd wordt.

De directie van de Hamboskliniek in Kerkrade heeft de zorgverleners in het verpleeghuis gevraagd om extra voorzichtig te worden bij het verzorgen van patiënten. De huidige maatregelen moeten de besmetting voorkomen, maar de directie van de Hamboskliniek is bang dat de besmetting kan voortduren als er geen extra voorzichtigheid wordt getoond.

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