

Contamination of the cold water distribution system of health care facilities by *Legionella pneumophila*: Do we know the true dimension?

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German water guidelines do not recommend routine assessment of cold water for *Legionella* in healthcare facilities, except if the water temperature at distal sites exceeds 25 °C. This study evaluates *Legionella* contamination in cold and warm water supplies of healthcare facilities in Hesse, Germany, and analyses the relationship between cold water temperature and *Legionella* contamination. Samples were collected from four facilities, with cases of healthcare-associated Legionnaires' disease or notable contamination of their water supply. Fifty-nine samples were from central lines and 625 from distal sites, comprising 316 cold and 309 warm water samples. *Legionella* was isolated from central lines in two facilities and from distal sites in four facilities. 17% of all central and 32% of all distal samples were contaminated. At distal sites, cold water samples were more frequently contaminated with *Legionella* (40% vs 23%, $p < 0.001$) and with higher concentrations of *Legionella* ($\geq 1,000$ colony-forming unit/100 ml) (16% vs 6%, $p < 0.001$) than warm water samples. There was no clear correlation between the cold water temperature at sampling time and the contamination rate. 35% of cold water samples under 20 °C at collection were contaminated. Our data highlight the importance of assessing the cold water supply of healthcare facilities for *Legionella* in the context of an intensified analysis.

Introduction

Legionnaires' disease (LD) is an important cause of hospital-acquired pneumonia [1]. Potable water was recognised as the major environmental source of healthcare-associated LD (hca-LD) in the early 1980s [1]. After this discovery, almost all cases of hca-LD have been linked to potable water [2-5]. For example, in the United Kingdom, 19 of 20 hospital LD outbreaks from 1980 to 1992 could be attributed to the water distribution system (WDS) [6]. Microaspiration is the major mode of transmission of hca-LD [7]. Because the clinical manifestations are non-specific, and specialised laboratory testing is required, LD is easily underdiagnosed [1,8].

Routine testing for *Legionella* of environmental water samples by culture has emerged as an effective strategy for prevention of hca-LD. Guidelines mandating routine monitoring of *Legionella* contamination of the WDS in hospitals and other healthcare facilities have been implemented in many European countries, including Spain, France, the United Kingdom, and Germany [1,9]. In contrast, the Centers for Disease Control and Prevention (CDC) recommends environmental cultures only when cases of hca-LD are discovered [10], an approach which remains controversial, taking into account that a specific diagnostic for LD is not routinely performed in many laboratories. For example, in the United States of America (USA) only 19% of the hospitals that participated in the CDC National Nosocomial Surveillance System did routinely provide *Legionella* testing of patients at high risk for developing hca-LD [11]. In Germany, the Federal Environment Agency (Umweltbundesamt) and the German National Public Health Institute (Robert Koch Institute) recommend periodical analysis of the WDS of hospitals, nursing homes and other healthcare facilities [12]. If a moderate to high level contamination is detected, i.e. at *Legionella* concentration of $\geq 1,000$ colony-forming unit (cfu)/100 ml, an intensified analysis with additional sampling points according to the guidelines of the German Technical and Scientific Association for Gas and Water (DVGW) is recommended [12,13].

Legionella can grow and amplify at temperatures between 25 °C and 45 °C with an optimum between 32 °C and 42 °C. *Legionella pneumophila* is able to withstand temperatures of 50 °C for several hours, but does not multiply at temperatures below 20 °C [9]. Therefore, keeping water temperature outside the range for *Legionella*, i.e. ≥ 55 °C and < 20 °C is an effective prevention and control measure for both warm and cold water systems. In Germany, which has a temperate climate, the temperature of cold water at entry to a building is usually below 20 °C. The German guidelines do not recommend routine assessment of cold water for *Legionella* contamination. In the context of intensified analysis, assessment of cold water is rec-

ommended if the water temperature at the distal site exceeds 25 °C [12].

The Hesse State Health Office (HSHO) is a federal institution in charge of surveillance, prevention, and control of LD in Hesse, a state with six million inhabitants located in west-central Germany. The diagnostic laboratories of HSHO offer a broad spectrum of chemical and microbiological analysis for water samples. Our institution is usually consulted by the communal health authorities when cases of hca-LD are detected in a healthcare facility or if routine environmental cultures reveal a notable contamination by *Legionella* species. We here present the results of the evaluation of the WDS of four healthcare facilities, which had contacted us for assistance to control and prevent *Legionella* contamination of their WDS. Two cases of hca-LD had been diagnosed in one facility, an acute care hospital with a solid organ transplantation unit, whereas a moderate to high *Legionella* contamination had been detected upon routine assessment in the other facilities, which included a rehabilitation centre and two nursing homes. A multidisciplinary team was sent to each facility in order to determine the extent of contamination of the WDS, to assess the contamination of cold and warm WDS independently and to investigate a possible correlation between the water temperature at sampling time and the extent of *Legionella* contamination.

Methods

Healthcare facilities

The healthcare facilities included in this study consisted of an acute care hospital specialised in thoracic surgery and solid organ transplantation (260 beds), a rehabilitation centre with cardiologic, orthopaedic and psychosomatic departments (183 beds), a nursing home for physically disabled individuals (47 beds), and a nursing home for elderly people (220 beds). These facilities had been requested by the Communal Health Office to conduct intensified *Legionella* monitoring because high *Legionella* concentrations had been detected during periodical assessment and/or cases of hca-LP had been reported. Each facility was visited by a team of specialists of the Communal Health Office and the HSHO several times (four to six times) between March 2009 and August 2010. The results presented in this study are derived from the analysis of samples

that were obtained at the first visit of our team to the facilities between March 2009 and February 2010.

Sampling procedure

Sampling points were selected by the team of specialists in cooperation with the technical teams of the facilities to obtain a comprehensive sample of cold and warm water for intensified analysis, in accordance with the recommendations of DVGW [13]. Fifty-nine samples were obtained from central lines (cold and hot-water tanks, return lines) of all facilities, including facility A (one warm sample), facility B (four cold samples), facility C (24 warm, 25 cold samples), and facility D (three warm, two cold samples). Six hundred and twenty-five samples were obtained from distal sites (467 showerheads, 155 taps, one pond and two spring fountains) of the facilities, comprising facility A (10 warm, 12 cold samples), facility B (15 warm, 16 cold samples), facility C (252 warm, 256 cold samples), and facility D (32 warm, 32 cold samples). Cold and warm water were generally sampled in parallel at distal sites. The temperature was documented and samples of approximately 200 ml were collected at central sites after discarding 3 L of cold or 3 L of warm water, and at distal sites after discarding 3 L of cold or 5 L of warm water, according to recommendations of the Federal Environment Agency [12]. It is noteworthy that the latter sampling method differs slightly from the European guidelines, which recommend samples of one litre in volume to be collected immediately after the opening of the water outlet [14].

Laboratory investigation

Legionella culture was performed on GVPC agar (Oxoid) according to recommendations of the Federal Environment Agency [15]. Two aliquots of 0.5 ml water were inoculated directly to GVPC agar and 100 ml was filtered through a 0.45 µm cellulose-nitrate membrane. The filter was overlaid with 20 ml 0.2 M HCl-KCl [pH 2.2] and incubated for 4–5 min. The buffer was discarded, the filter was rinsed with 10 ml sterile water and placed on GVPC agar. The cultures were incubated at 37 °C in a humidified atmosphere and examined after three, five, seven and 10 days. The detection limit of our method was one cfu/100 ml.

TABLE 1

Legionella contamination rate in cold and warm water samples obtained from four healthcare facilities, Hesse, Germany, March 2009–February 2010 (n=684)

Sample collection site	Sample type	<i>Legionella</i> positive n (%)	<i>Legionella</i> negative n (%)	Total n
Central line	All	10 (17)	49 (83)	59
	Cold water	1 (3)	30 (97)	31
	Warm water	9 (32)	19 (68)	28
Distal	All	197 (32)	428 (68)	625
	Cold water	125 (40)	191 (60)	316
	Warm water	72 (23)	237 (77)	309

Identification was conducted by performing subcultures of at least three colonies per sample on BCYE agar (Oxoid) and sheep-blood agar. *Legionella* isolates grew on BCYE agar but not on sheep-blood agar. Serotyping was performed with a latex agglutination kit (*Legionella* Latex Test, Oxoid), which allows the identification of *Legionella pneumophila* serogroup 1, *L. pneumophila* serogroups 2-14, and non-pneumophila *Legionella* species.

Statistical analysis

Statistical analysis was performed with Stata, Version 11.1, 2009 (StataCorp LP, Texas, USA). Chi square test

or Fisher exact test were used for analyzing qualitative data. Results were considered statistically significant when the P value was <0.05.

Results

Contamination rate in cold and warm water

Fifty-nine samples were collected at central lines, including 28 warm (temperature range: 46–75 °C) and 31 cold (temperature range: 7–14 °C) water samples. A total of 10 of 59 central samples were contaminated, comprising nine of 28 warm and one of 31 cold water samples (Table 1). Hence, among the central samples, warm water was more frequently contaminated with *Legionella* than cold water ($p<0.001$).

Six hundred and twenty-five distal samples were analysed, including 309 warm (temperature range: 32–70 °C) and 316 cold (temperature range: 7–29 °C) water samples. A total of 197 of 625 (32%) distal samples were contaminated. *Legionella* was detected in 125 of 316 (40%) cold water samples and 72 of 309 (23%) warm water samples (Table 1). Thus, among the distal samples, cold water was more frequently contaminated with *Legionella* than warm water ($p<0.001$).

We next evaluated the results at the level of individual facilities. The temperature of cold and warm water differed slightly between the facilities. At distal sites, cold water temperatures of 8–25 °C (facility A), 9–24 °C (facility B), 7–28 °C (facility C), and 13–29 °C (facility D) and warm water temperatures of 40–64 °C (facility A), 36–65 °C (facility B), 32–70 °C (facility C), and 50–66 °C (facility D) were measured at sampling time. *Legionella*

FIGURE 1

Legionella contamination in cold and warm water collected at distal sampling sites in four healthcare facilities, Hesse, Germany, between March 2009 and February 2010 (n= 625)

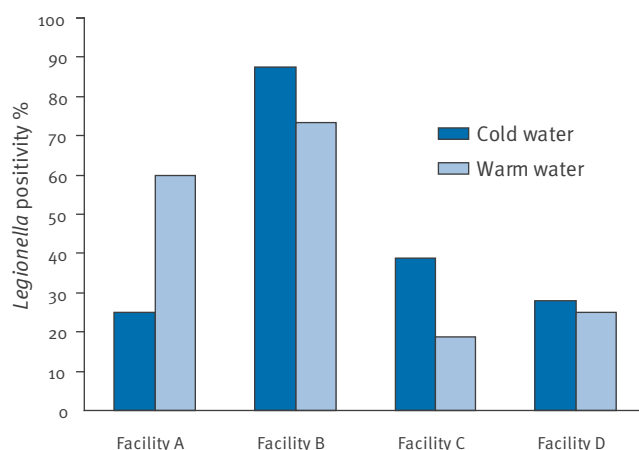


TABLE 2

Legionella contamination in distal cold and warm water samples collected in four healthcare facilities, Hesse, Germany, March 2009–February 2010 (n=625)

Healthcare facility	Cold water			Warm water		
	Total	<i>Legionella</i> positive	<i>Legionella</i> ≥1,000 cfu/100 ml	Total	<i>Legionella</i> positive	<i>Legionella</i> ≥1,000 cfu/100 ml
Facility A (n=22)	12	3	0	10	6	0
Facility B (n=31)	16	14	8	15	11	5
Facility C (n=508)	256	99	37	252	47	15
Facility D (n=64)	32	9	4	32	8	0

TABLE 3

Legionella concentration and temperature range of cold and warm water collected at distal sites in four healthcare facilities, Hesse, Germany, March 2009–February 2010 (n= 625)

<i>Legionella</i> concentration (cfu/100 ml)	Cold water			Warm water			P value ^a
	Temperature range (°C)	n	%	Temperature range (°C)	n	%	
<1	7–28	191	60	38–70	237	77	<0.001
1–99	8–25	13	4	39–65	18	6	0.361
100–999	11–27	63	20	37–64	34	11	0.003
≥1,000	11–29	49	16	32–62	20	6	<0.001
Total	7–29	316	100	32–70	309	100	

^a The P values were calculated by comparing the proportion of cold water samples displaying a distinct *Legionella* concentration among all cold water samples with the proportion of warm water samples with the similar *Legionella* concentration among all warm water samples.

contamination was detected in distal cold and warm water of all facilities. The overall positivity rate was nine of 22 (41%), 25 of 31 (81%), 146 of 508 (29%), and 17 of 64 (27%) in distal water of the facilities A, B, C, and D, respectively. Remarkably, contamination was more frequently detected in cold water than in warm water in three facilities (Figure 1). The contamination

rate of cold and warm water in the facilities A, B, C, and D were 25% versus 60%, 88% versus 73%, 39 versus 19%, and 28 versus 25%, respectively (Table 2).

Legionella species and serogroups detected

Serological differentiation of the *Legionella* isolates from the WDS revealed *L. pneumophila* serogroup 1 in facility A, C, and D, *L. pneumophila* serogroup 2-14 in facility B, and non-pneumophila *Legionella spp.* in facility A and C. *L. pneumophila* serogroup 1 was also isolated from the bronchoalveolar lavage fluid of the index patient with hca-LD in facility C. The *L. pneumophila* isolates obtained from the patient and the water supply displayed the same geno- and serotype, as determined by multilocus sequence typing (MLST) and monoclonal antibody serotyping, which were performed at the *Legionella* Reference Laboratory, University of Dresden, Germany.

Legionella concentration in cold and warm water

Of 316 distal cold water samples analysed, 60% were tested negative for *Legionella*, 4% revealed minimal contamination (colony count 1–99 cfu/100 ml), 20% moderate contamination (100–999 cfu/100 ml) and 16% high contamination ($\geq 1,000$ cfu/100 ml). Of 309 distal warm water samples analysed, 77% were negative, 6% displayed minimal contamination, 11% moderate contamination, and 6% high contamination (Table 3). In detail, a total of 69 samples comprising 49 cold and 20 warm water samples revealed a high *Legionella* concentration ($\geq 1,000$ cfu/100 ml). Thirty three of 49 (67%) highly contaminated cold water samples displayed a temperature of < 20 °C at collection time, whereas three of 20 (15%) highly contaminated warm water samples displayed a temperature of ≥ 55 °C at sampling time. Together, cold water samples were more frequently contaminated with higher *Legionella* concentrations compared to warm water samples. The difference between cold and warm water was significant in all categories except for minimal contamination (Table 3).

We next evaluated the prevalence of high *Legionella* concentrations, i.e. $\geq 1,000$ cfu/100 ml, in cold and warm water of different facilities. As shown in Table 2, a high grade contamination was detected in three of four facilities. Cold water samples were more frequently contaminated with high *Legionella* concentrations than warm water samples in three of four facilities (Table 2).

Relationship between temperature and Legionella contamination

We next examined the relationship between the temperature of distal water at sampling time and *Legionella* contamination. Cold and warm water samples were assigned to four groups, cold water < 20 °C, cold water ≥ 20 °C, warm water < 55 °C, and warm water ≥ 55 °C and the contamination rate was calculated for each group. The positivity rate was 94 of 265 (35%), 31 of 51 (61%), 45 of 52 (87%), and 27 of 257 (11%) in the latter groups, respectively (Figure 2). It is noteworthy that 35% of

FIGURE 2

Relationship between the temperature of distal water at sampling time and *Legionella* contamination, Hesse, Germany, March 2009–February 2010 (n= 625)

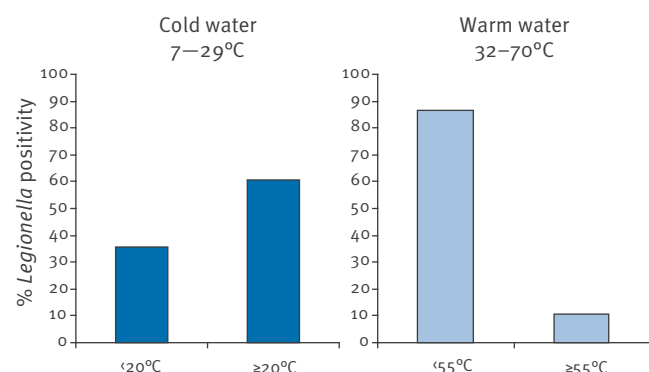
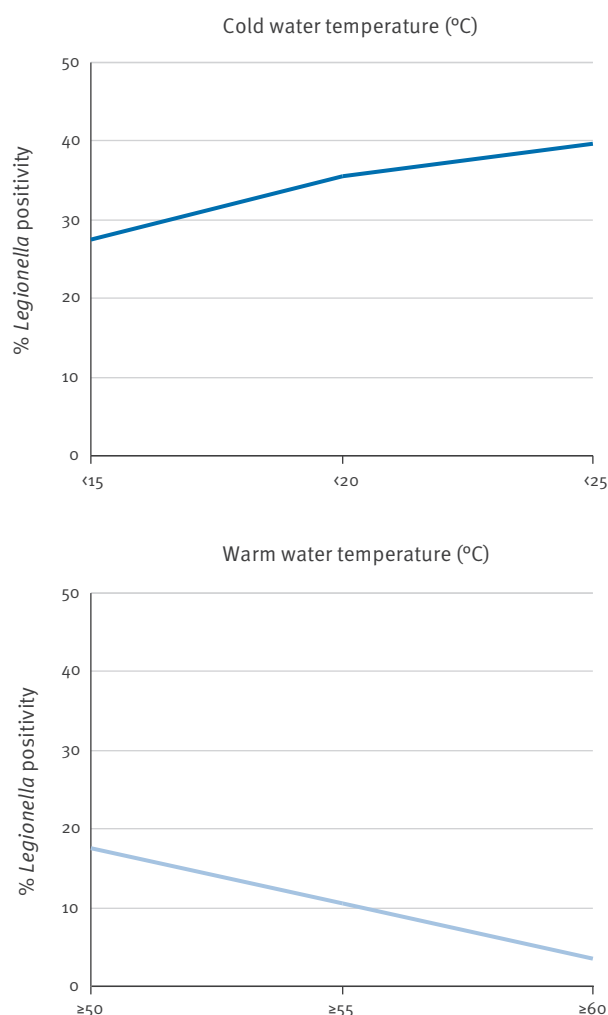


FIGURE 3

Relationship between contamination rate of distal water and the threshold temperature for cold and warm water, Hesse, Germany, March 2009–February 2010 (n= 625)



cold water samples that displayed an optimal temperature in terms of *Legionella* prevention at sampling time, that is <20 °C, were contaminated. In contrast, only 11% of warm water samples that displayed an optimal temperature in terms of *Legionella* prevention, that is ≥55 °C, were contaminated. Outside the temperature range of *Legionella* growth, there was significantly less contamination in warm water than contamination in cold water ($p < 0.001$).

We further examined whether we may find a threshold temperature that would allow a reliable discrimination between contaminated and non-contaminated distal water. The threshold temperatures of 15 °C, 20 °C and 25 °C were tested for cold water, and 50 °C, 55 °C, and 60 °C for warm water. The contamination rate of samples beyond the selected temperature was calculated separately. As shown in Figure 3, 43 of 156 (28%) of water samples that were below 15 °C at sampling time, which is below the lower limit (20 °C) of the range of *Legionella* growth, were contaminated by *Legionella*. This suggests that measuring cold water temperature at sampling does not allow the defining of a reliable temperature threshold, below which cold water would be considered free from *Legionella* contamination.

Discussion

We here present the results of assessment of the water supplies of four healthcare facilities in Germany. The investigation was initiated because cases of hca-LD were diagnosed in one facility (Facility C) or because periodical analysis had suggested a severe contamination of the WDS with *Legionella* (facilities A, B, and D). The contamination rate of distal water samples was 41%, 81%, 29% and 27% in the four facilities examined. The very high rate in some cases (81%) was not entirely unexpected in light of the circumstances that had led to the enrolment of the facilities in this study.

We found higher contamination rates and higher *Legionella* concentrations in cold water samples than in warm water samples collected from distal sites in three facilities (Figure 1, Table 2). Legionellosis has been traditionally associated with inadequately heated warm water [1]. There is a common belief that only the warm water supply may serve as a source of infection. Nonetheless, previous studies have shown that the cold water supply of healthcare facilities may be heavily contaminated with *Legionella* species [16]. Other investigators have reported cases of hca-LD that were attributed to contamination of the cold water supply. Hoebe et al. [17] reported two cases of fatal LD in a rehabilitation centre linked to the cold water supply. Johansson et al. [18] described a case of hca-LD in Sweden that was clearly linked to the cold WDS. Graman et al. [19] reported a case of hca-LD that was traced back to a contaminated ice machine. Our data show that the cold water supply of healthcare facilities may be even more heavily contaminated by *Legionella* species than the warm water supply. We found *Legionella* concentrations of up to 10,000 cfu/100 ml in distal cold water samples

(data not shown). Different factors may have contributed to this interesting phenomenon. It is possible that a thermal disinfection of warm WDS was performed shortly prior to our visit to the facility. This could have resulted in a temporal suppression of *Legionella* in the warm water supply. Another possible explanation is a “warming-up” of cold water, which may occur after long intervals of stasis or when the cold and warm water pipes are closely fitted in the same shaft and run together over a long distance without appropriate insulation. The warming-up effect may not be detectable at the time of sampling, which is usually during daytime on a weekday. In the latter case, hot water flushing of warm water tubes may even have a paradoxical effect on contamination of the cold WDS by aggravating the warming-up effect.

Analysis of the temperature of distal samples revealed that only 16 of 316 (5%) cold water samples displayed a temperature of 25 °C or more at sampling time, which is the threshold temperature recommended by the German water guidelines for assessment of cold water [12]. We therefore tested other threshold temperatures. We found that 94 of 265 (35%) and 43 of 156 (28%) of the distal cold water samples that displayed a temperature of <20 °C and <15 °C at sampling time were contaminated (Figure 3). Taken together, our data show that high *Legionella* concentrations may be found in cold water samples displaying a temperature of as low as 11 °C at sampling time, whereas no or very low *Legionella* concentrations may be associated with cold water temperatures of up to 28 °C at sampling time (Table 3). Hence, our data suggest that there is no reliable correlation between the temperature of cold water at sampling time and the extent of *Legionella* contamination. A possible explanation for this incoherence is that the temperature at sampling time, which is usually a busy time on a working day, is not representative of the temperatures that the sampled water has undergone prior to sampling.

After release of the results of our investigation, the infection control precautions were reassessed in all facilities and additional decontamination measures and prevention strategies were initiated for the warm and cold WDS. The results of the intervention activities were controlled by follow-up investigation.

In conclusion, our data suggest that the cold water supply of healthcare facilities may be heavily contaminated with *Legionella* species. We did not find a reliable correlation between cold water temperature at sampling time and *Legionella* contamination rate or concentration. If we had restricted our analysis to cold water samples that displayed at least 25 °C at sampling time, we would have missed many cases of severe contamination. Our results highlight the importance of assessment of cold water in the context of intensified analysis of the water supply of healthcare facilities.

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