



Occurrence of Legionella in UK household showers

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ABSTRACT

Household water systems have been proposed as a source of sporadic, community acquired Legionnaires' disease. Showers represent a frequently used aerosol generating device in the domestic setting yet little is known about the occurrence of *Legionella* spp. in these systems. This study has investigated the prevalence of *Legionella* spp. by culture and qPCR in UK household showers. Ninety nine showers from 82 separate properties in the South of England were sampled. Clinically relevant *Legionella* spp. were isolated by culture in 8% of shower water samples representing 6% of households. *Legionella pneumophila* sg1 ST59 was isolated from two showers in one property and air sampling demonstrated its presence in the aerosol state. A further 31% of showers were positive by *Legionella* spp. qPCR. By multi-variable binomial regression modelling *Legionella* spp. qPCR positivity was associated with the age of the property ($p=0.02$), the age of the shower ($p=0.01$) and the frequency of use ($p=0.09$). The concentration of *Legionella* spp. detected by qPCR was shown to decrease with increased frequency of use ($p=0.04$) and more frequent showerhead cleaning ($p=0.05$). There was no association between *Legionella* spp. qPCR positivity and the cold water supply or the showerhead material ($p=0.65$ and $p=0.71$, respectively). Household showers may be important reservoirs of clinically significant *Legionella* and should be considered in source investigations. Simple public health advice may help to mitigate the risk of *Legionella* exposure in the domestic shower environment.

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1. Introduction

Legionellae are the causative agents of Legionnaires' disease (LD), a potentially fatal pneumonia and Pontiac fever, a milder self-limiting illness (Phin et al., 2014). They are ubiquitous in aqueous environments but favour growth in man-made water systems operating between 20 °C and 45 °C (Fields et al., 2002). World-wide, the incidence of LD is increasing with the highest number of cases ever reported in Europe in 2014 (European Centre for Disease Control and Prevention, 2016). A wealth of epidemiological evidence unambiguously links LD to the inhalation of aerosols from contaminated water (Bartram et al., 2007). Whilst cooling towers and spa pools are well-documented causes of large outbreaks (Bennett et al., 2014; Den Boer et al., 2002) most LD cases are sporadic and acquired in the community (i.e. not acquired in hospitals or during travel abroad). For the majority of these cases an environmental source is never found (van Heijnsbergen et al., 2015). Household potable water systems have been shown to be a potential source of sporadic LD (Raw et al., 2000; Straus et al., 1996; Verhoef et al., 2004). Showering has

become more popular than bathing (Hand et al., 2003) and showers likely represent the most frequently used aerosol-generating device in the household setting. Exposure to *Legionella* contaminated showers is a recognised risk factor for Legionellosis (Bauer et al., 2008; Muhlenberg, 1993; Tobin et al., 1980) and previous exposure assessment has ranked showers second (behind ultrasonic and cool mist humidifiers) in a relative ranking of *Legionella* exposure pathways from common household water uses (Hines et al., 2014). However, there is limited information on the prevalence of *Legionella* in household showers and the associated risk to users. With aging and increasingly immunocompromised populations (Chan et al., 2016) a better understanding of opportunistic pathogens including *Legionella* in household water systems will become more important. This study is the first investigation of the prevalence of *Legionella* spp. in UK household showers.

2. Materials and methods

2.1. Sampling sites

Households were located in 11 counties and mainly clustered around the cities and surrounding areas of Bath, Bristol, Oxford, Portsmouth, Salisbury and Southampton.

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2.2. Water and swab sampling

Residents collected the first litre water sample, after several hours of shower inactivity, made up of the standing water in the shower armature as well as water from adjacent pipelines, in sterile bottles (TSC, Heywood, UK) containing sodium thiosulphate (20 mg/L). Showers were set to the temperature normally used by the resident. Where possible, swab samples were taken from the inner surface of flexible hoses using sterile cotton swabs (TCS) placed in 4 ml sterile distilled water. The age of the property, age of the shower, type of shower (electric or manual), showerhead material (plastic or metal), hot water supply (combination boiler or hot water storage tank), cold water supply (storage tank or mains), temperature of hot water storage/generation, shower head cleaning frequency (never, weekly, monthly, quarterly or yearly) and frequency of use (showers/week) were recorded. Samples were transported to the laboratory and processed within 24 h.

2.3. Detection of *Legionella* by culture and qPCR

Two aliquots (300 ml) of water samples were concentrated by membrane filtration. One aliquot was cultured for *Legionella* according to ISO 11731:1998. Briefly, bacteria were re-suspended in 10 ml 1/40 'Ringers' solution. Sample concentrates (500 µl) were plated onto Glycine-Vancomycin-Polymyxin-Cycloheximide (GVPC) agar (Thermofisher Scientific, Basingstoke, UK) either directly (untreated), or after additional heat (30 min at 50 °C) or acid (0.2 mol l⁻¹ HCl-KCl, pH2.2 for 5 min) treatments. Plates were incubated at 36 ± 1 °C for 10 days. Presumptive *Legionella* isolates were sub-cultured onto buffered charcoal yeast extract agar without l-cysteine (BCYE-, Oxoid) and GVPC. The other aliquot was analysed by qPCR for *Legionella* spp., *L. pneumophila* and *L. pneumophila* serogroup 1 (LP1) according to ISO TS 12869:2012 using a previously described assay (Collins et al., 2015) with the following modifications: DNA was extracted using the PowerWater DNA isolation kit (MoBio, Carlsbad, USA) as per the manufacturer's instructions and TaqMan[®] Fast Environmental Master Mix Beads (Thermofisher Scientific) incorporating a VIC 3' labelled internal positive control were used for all qPCR reactions. Data were analysed using the ABI 7500 v2.3 Software (Thermofisher Scientific). Swabs were shaken at 1500 rpm (10 min) with glass beads. Culture and qPCR were performed as above. The limits of detection for culture were 6.6 × 10¹ CFU/L and 1.6 × 10¹ CFU/swab. The limits of detection and quantification for qPCR were 1.6 × 10² genome units (GU)/L and 2.0 × 10² GU/swab and 3.3 × 10² GU/L and 4.0 × 10² GU/swab, respectively.

2.4. Identification of culture isolates

Presumptive *Legionella* isolates exhibiting cysteine auxotrophy were identified by matrix assisted laser desorption and ionisation time of flight mass spectrometry (MALDI-ToF, (Bruker, Coventry, UK)) using the full extraction method and latex agglutination (Thermofisher Scientific) according to the manufacturer's protocols. Isolates not identifiable by MALDI-ToF were identified by *Mip* gene sequencing (Ratcliff et al., 1998). LP1 isolates were sequence typed according to an internationally validated sequence-based typing (SBT) scheme (Gaia et al., 2005; Ratzow et al., 2007). Results of *Mip* gene sequencing and SBT were analysed using the online Sequence Quality Tools (Public Health England, 2016a, 2016c).

2.5. Air sampling

To investigate the aerosolisation of *Legionella* from shower water a six-stage viable Andersen sampler (Andersen, 1958), operating at a flow rate of 28.3 l/min sampling onto GVPC plates and

an all-glass cyclone sampler (Upton et al., 1994) operating at a flow rate of 650 l/min collecting into phosphate buffered mannitol (PBMA) were used to collect aerosols from showers. Samplers were positioned 1 m from the shower at a height of 1.6 m with the doors and windows closed. Each shower was sampled twice starting at the time of shower activation for a period of five and 10 min. The GVPC plates were incubated for 10 days at 36 ± 1 °C. Cyclone concentrates were cultured and processed for qPCR according to the method for water samples. Relative humidity and temperature were recorded during sampling. The theoretical limits of detection for air sampling were 7 CFU/m³ and 3.5 CFU/m³ for five and 10 min Andersen sampling, 3 CFU/m³ for cyclone sampling and 3.1 × 10¹ GU/m³ for qPCR.

2.6. Statistical analysis

Statistical analysis was conducted using STATA v14. Multivariable binomial regression modelling with a logarithmic link function was used to estimate proportion positivity ratios. Poisson regression models were used to obtain positivity ratios when binomial models failed to converge. For *Legionella* spp. concentration (GU/L) a censored regression analysis was performed after taking natural logarithms of the GU/L values.

3. Results and discussion

3.1. Sampling sites

Ninety nine showers from 82 households were sampled. The majority of households (79/82, 96.3%) were single-family occupancy houses whilst three households were flats in multi-occupancy buildings. Properties were aged between 0.5 and 136 years (median 56 years) and showers between 0.5 and 34 years (median 6 years). The majority of showers were manual mixers of which 41.9% were supplied with hot water from combination boilers and 55.4% from hot water storage tanks (HWST) (Table 1). Cold water was supplied from the mains for 72.7% showers and from cold water storage tanks for 23.2%.

3.2. Detection of *Legionella*

Legionella spp. were cultured in 8.1% of water samples and 1.1% of swabs representing 6.1% of households (Table 2). This isolation rate is lower than those reported in other countries including Canada (12.4%) (Alary and Joly, 1991) and Italy (22.6%) (Borella et al., 2004). *Legionella pneumophila* serogroup 1 was isolated in two water samples from the same household (household 1) at concentrations of 1 × 10³ CFU/L and 1.3 × 10⁴ CFU/L. Sequence-based typing of multiple isolates from both showers identified them as sequence type (ST) 59. This ST has previously been associated with human disease with 68 clinical entries in the international database (Public Health England, 2016c) (accessed October 26 2016). One additional shower was positive for LP1 by qPCR only (6.0 × 10² GU/L). Three clinically relevant *Legionella* spp. were isolated from four additional households; *Legionella anisa*, from two, *L. micdadei* from one and *L. steelei* from three showers in the same household (household 2). To our knowledge this is the first reported isolation of *L. steelei* in the UK.

All culture positive water samples were positive for *Legionella* spp. by qPCR and qPCR was more sensitive than culture detecting a further 31/99 (31.3%) positive water samples. Differences between culture and qPCR results are explained in detail elsewhere (Dusserre et al., 2008; Joly et al., 2006) but include the detection of viable but non-culturable cells, cells inside amoeba and *Legionella* spp. that are difficult to culture under standard laboratory

Table 1

Characteristics of the showers sampled. Manual showers included three pump-assisted thermostatic showers. *Data for manual showers only. Two residents were unsure where the hot water was supplied from. † Four residents were unsure where the cold water was supplied from. HWST, hot water storage tank. CWST, cold water storage tank.

	Shower type		Hot water supply*		Cold water supply†	
	Manual	Electric	HWST	Combi boiler	CWST	Mains
No of showers (%)	74 (74.7)	25 (25.3)	41 (55.4)	31 (41.9)	23 (23.2)	72 (72.7)

Table 2

Culture and qPCR analysis for water and swab samples. CFU, colony forming units; GU, genome units.

	Positive water n (%)	CFU/L or GU/ L (Median)	Positive swab n (%)	CFU/swab or GU/ swab (Median)	Positive households (%)
Culture	8/99 (8.1)	$4.0 \times 10^1 - 1.3 \times 10^4$ (1.1×10^3)	1/87 (1.1)	5.4×10^2	5/82 (6.1)
<i>Legionella</i> spp. qPCR	38/99 (38.4)	$<3.3 \times 10^2 - 1.2 \times 10^6$ (1.2×10^4)	16/87 (18.4)	$6.8 \times 10^2 - 1.7 \times 10^6$ (6.6×10^3)	39/82 (48)
LP1 qPCR	3/99 (3.3)	$6.0 \times 10^2 - 1.4 \times 10^4$ (4.0×10^3)	0/87	N/A	2/82 (2.4)

conditions (Lee et al., 1993). There were no qPCR-negative-culture-positive samples, representing a qPCR negative predictive value of 100%. In total, 48% of households had one or more *Legionella* qPCR positive shower. Of the 12 households with multiple showers sampled, a total of five (42%) had all showers positive and three (25%) had two out of three positive. Only 15/38 (40%) positive shower water samples had corresponding positive swab samples. Although only a small section of the shower hose was swabbed this could suggest the absence of a *Legionella* biofilm in the hose and indicate contamination had originated further upstream.

Further investigation of households 1 and 2 revealed that both properties stored hot water $>60^\circ\text{C}$ and distributed it $\geq 55^\circ\text{C}$. Cold water was supplied or stored at $<20^\circ\text{C}$. These temperatures are generally considered suitable for *Legionella* control (Bartram et al., 2007). Household 1 had two pump-assisted thermostatic showers supplied by a CWST and a hot water storage tank (HWST) whilst household 2 had three manually operated mixer showers fed by the mains and a HWST. Additional hot and cold water outlets in both households were negative for *Legionella* spp. by both culture and qPCR indicating a local contamination of the showers and/or upstream pipework and not a systemic colonisation of the water system. This is an interesting observation. Risk assessors should take into consideration that systems that appeared to have parameters in place that should have minimised the growth of *Legionella* ($>55^\circ\text{C}$, $<20^\circ\text{C}$, mains fed showers and good frequency of use) were in fact positive for *Legionella* including LP1.

Six culture positive showers, including those in households 1 and 2, and two qPCR negative showers were resampled after a period of two months. *Legionella* continued to be detected by both culture and qPCR (at similar concentrations to those detected previously) from three positive showers including both showers in household 1, suggesting contamination had remained relatively stable. The three showers in household 2 were negative by both culture and qPCR upon resampling, indicating potential transient contamination. Both showers that were originally negative by qPCR were consistently negative.

3.3. Variables associated with the prevalence of *Legionella* spp.

Accounting for confounding factors, multiple binomial regression modelling provided evidence that several variables were associated with *Legionella* qPCR positivity. These are summarised in Table 3. Positivity was low in newer built properties (2/11, 18% positive for properties ≤ 10 years) but increased rapidly to a maximum of approximately 65% in homes built around 30 years

Table 3

Variables associated with *Legionella* spp. positivity by qPCR from the multiple variable binomial regression analysis. *: cannot be used directly as estimated positivity ratios.

Variable	Estimated positivity ratio	95% CI	P value
Property age (pa)			
log(pa)	0.687*	0.463 to 1.019	0.02
log(pa) ²	0.579*	0.386 to 0.868	
Shower age (sa)			
log ₂ (sa)	1.408	1.076 to 1.843	0.01
Frequency of use			
Showers/week	0.974	0.945 to 1.005	0.09
Hot water supply			
HWST	reference		
Combi boiler	0.599	0.324 to 1.106	0.10

ago (Fig. 1). There was a steady decline in positivity in properties older than 30 years. It is not possible to determine factors related to higher positivity in 30 year old properties however, they could include insufficient time for *Legionella* colonisation in newer properties, differences in water pipe material (older properties are more likely to use copper pipes as opposed to plastic pipes) and improvements in modern building/water system design that may be protective against colonisation. Future studies should examine potential factors and household age may need to be considered in risk assessments.

Legionella spp. qPCR positivity was low in newer showers (2/19, 10.5% in showers ≤ 2 years of age), but increased to an estimated 56% in showers ≥ 10 years. There was an estimated 43% (95% CI 14.5% to 78.6%) increase in positivity for a doubling of shower age suggesting that *Legionella* contamination may be time dependent. A log-linear relationship between frequency of use and *Legionella* positivity was observed (Fig. 1) indicating increased shower use is protective against positivity. There were 28 showers that were used less than once a day on average of which 15 (53.6%) were positive. For each additional use of a shower per week the estimated positivity decreased by an estimated 4.6% (95% CI 0.4%–8.1%). Hot water supplied to the shower by an instantaneous combination boiler as opposed to the storage of hot water was also shown to be weakly protective (Table 3) with an estimated 55.3% (95% CI 12.4%–77.1%) reduction in positivity. These findings are consistent with the knowledge that stagnant water, low turnover and storage of hot water are recognised risk factors for *Legionella* colonisation and proliferation (Bartram et al., 2007). Although cold water sup-

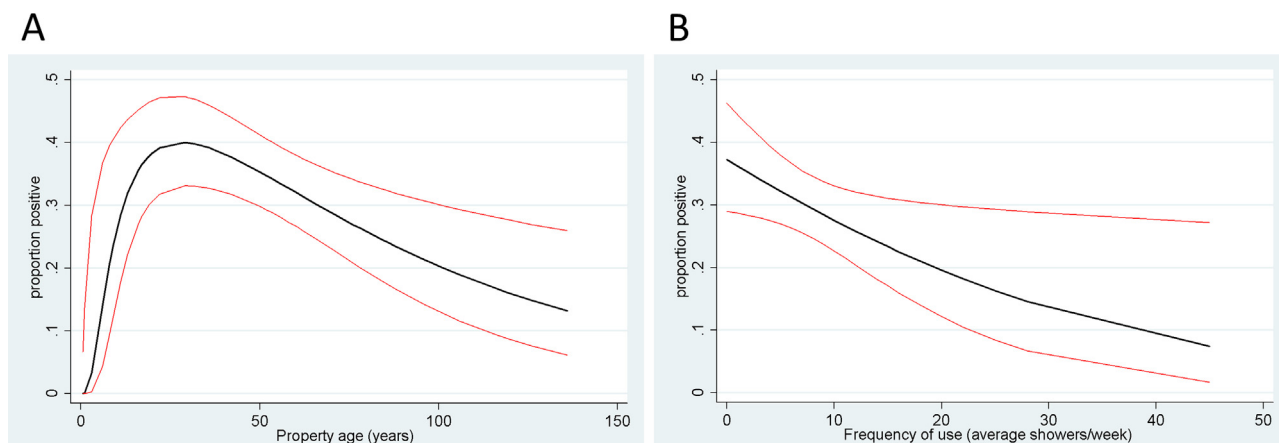


Fig. 1. Modelled relationship between proportion *Legionella* spp. qPCR positive and the age of the property (A) and the frequency of use (B). The black lines represent the estimated regression and the red lines indicate the 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4

Variables significantly associated with *Legionella* concentration (GU/L) from the single variable censored regression analysis. GU, genome units.

Variable	Estimated relative mean GU/L	95% CI	P value
Shower age Per year	1.408	1.076 to 1.843	0.01
Frequency of use Showers/week	0.917	0.852 to 0.998	0.04
Cleaning frequency			
Never	reference		
Weekly	0.202	0.036 to 1.122	
Monthly	0.075	0.012 to 0.470	
Quarterly	0.489	0.095 to 2.511	
Annually	1.812	0.275 to 11.95	0.05

ply from the mains was shown to be weakly protective against *Legionella* positivity ($p = 0.16$) it was also highly associated with the use of a combination boiler (Fishers exact test, $p = 0.002$). Adjusting for this association, the cold water supply was shown not to be statistically significant ($p = 0.65$). This is contrary to a previous study (Raw et al., 2000) that found *Legionella* colonisation in domestic water systems was strongly associated with the cold water supply. There was no association between *Legionella* positivity and showerhead material ($p = 0.71$) nor hot water generation/storage temperature, although data for this variable were only available for 38/82 (46.3%) households. Shower age, frequency of use and cleaning frequency demonstrated a significant association with the concentration of *Legionella* detected by qPCR. Increasing shower age was associated with increased GU/L concentration (Table 4). Similar to the data for positivity rates, the frequency of use was shown to reduce the GU/L detected with the highest concentration (1.2×10^6 GU/L) detected in a shower used less than once per week and the lowest concentration ($<3.3 \times 10^2$ GU/L) detected in a shower used 21 times per week. Eighteen of 39 (46%) *Legionella* positive showers were never cleaned by the resident. Increased cleaning frequency was weakly associated with the concentration of *Legionella* (GU/L) with an estimated relative mean of 1.8 for annual cleaning and 0.2 for weekly cleaning (Table 4).

Electric showers are generally considered lower risk for *Legionella* contamination as they produce hot water instantaneously without storage (Oliveira et al., 2007) thus the opportunity for *Legionella* to proliferate at a favourable temperature is reduced. Nonetheless, 11/25 (44%) electric showers in this study were positive for *Legionella* spp. (3/25, 12% by culture). Seven of the 11 (63.6%)

positive electric showers were fed directly from the mains which, although not statistically protective in this study, is traditionally thought to reduce the likelihood of *Legionella* contamination compared to the use of CWSTs (Raw et al., 2000). A further examination of some of these electric showers to determine precise areas of contamination would have been advantageous but not possible in this study. These findings suggest that electric showers, particularly in the domestic environment, should not be considered lower risk for contamination compared to manually operated showers. This warrants further research.

3.4. Air sampling

Air sampling was conducted at household 1 to investigate the aerosolisation of LP1. Relative humidity and temperature ranged between 88%–95% and 26.8 °C–33 °C respectively. Viable *Legionella* spp. were not cultured from the air by either Andersen or Cyclone samplers. LP1 was detected in the air by qPCR from the shower that had previously been found to have a concentration of 1.3×10^4 CFU/L. The concentration detected was 7.7×10^1 GU/m³. Concurrently with this study, a *L. anisa* positive shower (4×10^4 CFU/L) was identified in a workplace. Air sampling of that shower also failed to yield viable *Legionella* but LP1 (and no other *Legionella* spp.) was detected by qPCR (2.8×10^2 GU/m³) for one run only. This is an interesting finding as the shower water had not previously been positive for LP1 suggesting contamination originated upstream (e.g. biofilm sloughing) during the sampling period. Water sampling from both showers one week after air sampling confirmed that they were still positive by culture ($>1 \times 10^4$ CFU/L) for LP1 and *L. anisa*, respectively. It is noteworthy that the *Legionella* concentrations detected in the air by qPCR in this study fall within the low and best estimate predictions of critical air-borne *Legionella* necessary to result in infection (Schoen and Ashbolt, 2011). The inhaled dose of a person in a shower room can be calculated as the exposure time x concentration x breathing rate. For household 1, if we assume a 10 min exposure time, a concentration of 77 GU/m³ and a breathing rate of 0.02 m³ per min we get an estimated inhaled dose of 15.4 GU. The poor recovery of viable *Legionella* from aerosols is consistent with previous reports (Deloge-Abarkan et al., 2007; Dennis et al., 1984). The water chemistry and the stress of aerosolisation and sampling can reduce the recovery of viable organisms (Chang and Hung, 2012). It has been shown that a high concentration of *Legionella* (>300 CFU/ml) in shower water is required to detect *Legionella* from the air (Wiik and Krovel, 2014) which may explain why air-borne *Legionella* was infrequently detected

at low concentrations in this study. The low frequency of recovery of viable *Legionella* from the air may be a factor in the sporadic nature of the disease. Further studies are required to determine a standardised sampling methodology for the detection of *Legionella* in aerosols and to determine the risk posed by *Legionella* colonised showers. Controlled and reproducible experiments should be performed to robustly determine where *Legionella* colonisation occurs (which components are susceptible), emission factors – the relationship between contamination in the water and the amount of respirable *Legionella* dispersed into aerosols, aerosol partitioning and the persistence of air-borne *Legionella* from different types of showers. Such data would inform quantitative risk assessments and could be used to determine additional control measures to reduce exposure.

3.5. Limitations

A number of limitations have been identified for this study including the geographical spread of households. Regional differences may exist in terms of *Legionella* positivity. For the majority of properties, sampling occurred once therefore it was not possible to accurately determine if *Legionella* positivity was sustained or transient. However, with the exception of household 2, data from properties sampled on multiple occasions showed that *Legionella* positivity and negativity were relatively stable over a period of at least two months. Due to practical reasons, swabs were only taken from the flexible shower hoses. Previous studies have shown enrichment of opportunistic pathogens in showerheads (Feazel et al., 2009). This may explain why 40% of *Legionella* positive showers were only positive in the water and not from swabs. Furthermore, although viable *Legionella* spp. were detected in 6% of households, it is important to note that qPCR does not differentiate between viable and non-viable organisms therefore, it is not possible to determine if the *Legionella* detected by qPCR had the potential to cause disease. Finally, whilst this study has revealed a considerable prevalence of *Legionella* spp. in household showers, further studies are needed to elucidate the clinical significance of these findings. In the UK, households are not routinely sampled during investigations of sporadic LD unless a clinical respiratory specimen from the case has been epidemiologically typed to provide a comparison for any positive environmental isolates (Public Health England, 2016b). As a consequence, little data exists to indicate the public health burden posed by household water systems. Further difficulty is introduced by the assumed under-ascertainment of LD caused by Legionellae other than LP1 (von Baum et al., 2008).

4. Conclusions

This study has demonstrated that UK domestic showers are frequently (48%) positive for non-pneumophila *Legionella* including clinically relevant species. LP1, the causative agent of the majority of LD (Bartram et al., 2007) was detected less frequently (2.4% of households) but sampling showed that it could be detected in the air surrounding a contaminated shower. Domestic showers may be important reservoirs of *Legionella* infection and should be considered as such during source investigations. The lack of hot water storage, increased frequency of shower use and more frequent showerhead cleaning were shown to be protective against *Legionella* positivity and could be simple public health advice measures that may reduce the risk of exposure to *Legionella* from domestic showers. Understanding the prevalence of *Legionella* and associated exposure risks in the domestic environment should be a priority in light of an increasingly elderly and immunocompromised population.

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