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10 Questions

Ten questions concerning the aerosolization and transmission of *Legionella* in the built environment

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ABSTRACT

Legionella is a genus of pathogenic Gram-negative bacteria responsible for a serious disease known as legionellosis, which is transmitted via inhalation of this pathogen in aerosol form. There are two forms of legionellosis: Legionnaires' disease, which causes pneumonia-like symptoms, and Pontiac fever, which causes influenza-like symptoms. *Legionella* can be aerosolized from various water sources in the built environment including showers, faucets, hot tubs/swimming pools, cooling towers, and fountains. Incidence of the disease is higher in the summertime, possibly because of increased use of cooling towers for air conditioning systems and differences in water chemistry when outdoor temperatures are higher. Although there have been decades of research related to *Legionella* transmission, many knowledge gaps remain. While conventional wisdom suggests that showering is an important source of exposure in buildings, existing measurements do not provide strong support for this idea. There has been limited research on the potential for *Legionella* transmission through heating, ventilation, and air conditioning (HVAC) systems. Epidemiological data suggest a large proportion of legionellosis cases go unreported, as most people who are infected do not seek medical attention. Additionally, controlled laboratory studies examining water-to-air transfer and source tracking are still needed. Herein, we discuss ten questions that spotlight current knowledge about *Legionella* transmission in the built environment, engineering controls that might prevent future disease outbreaks, and future research that is needed to advance understanding of transmission and control of legionellosis.

1. Introduction

Legionella is a pathogenic Gram-negative bacterium and the causative agent of a disease known as legionellosis [1–4]. Legionnaires' disease and the less severe form, Pontiac fever, are the two most frequent presentations of legionellosis. The common clinical symptoms of legionellosis include high fever, cough, chills, difficulty breathing, neurological problems, muscle weakness, diarrhea, chest pain, headache, nausea, and vomiting [5]. Case-fatality rates of legionellosis, primarily Legionnaires' disease, fall in the range of ~10–50% depending on the specific outbreak [6]. Information regarding Pontiac fever is limited since many people who contract it do not seek medical attention, possibly due to the similarity in symptoms between this condition and other mild upper respiratory tract infections [7].

Legionella is a unique pathogen due to its water-to-air transmission route. Typically, the bacterium resides and grows in water systems (including as a biofilm on pipes), but it must be aerosolized and inhaled or aspirated to cause disease. Recently, partly motivated by increased incidence, many questions have arisen regarding *Legionella* transmission in the built environment and interventions that may be able to reduce the number of legionellosis cases in the future. We present ten questions and answers about *Legionella*, legionellosis etiology, and engineering controls with the potential to thwart disease transmission. Additionally, we discuss some of the major knowledge gaps that merit further research.

Q1) What is legionellosis, and how is it transmitted?

Legionellosis is an infection caused by gram-negative bacteria of the *Legionella* genus, named after an outbreak occurring at an American Legion convention in Philadelphia in 1976 [3]. Twenty-four of 58 [8] known *Legionella* species have been implicated in human disease. These highly diverse [9] and pleomorphic [10]

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bacteria can be found in a variety of environments and are naturally occurring in freshwater [9] and soil [11]. As their life cycle typically involves endoparasitization and replication within host eukaryotes such as amoebae [12], *Legionella* possess mechanisms to infect human cells, notably macrophages [8].

Legionellosis is a relatively common water-based disease, responsible for more drinking-water-related outbreaks in the United States than all other sources combined [13], although underreporting, particularly outside of outbreaks, is presumed to be common [14]. Estimates of legionellosis incidence in the United States range from 8,000 [15] to 50,000 cases per year [16], although reported case rates vary greatly by region [17]. The vast majority of cases are caused by one strain of the species *L. pneumophila*, serogroup 1 [6]. Strains responsible for outbreaks appear to be even more specific, with 85% being linked to a subset of serogroup 1 *L. pneumophila*, those belonging to the monoclonal antibody group 2 [18]. The physiological and ecological reasons for the increased virulence of these strains are complex, although specific virulence-related proteins have been identified, such as the O-antigen for serogroup 1[19] and the *mip* gene in *L. pneumophila* [20]. Despite the attention generated by clusters of legionellosis, outbreaks are responsible for a small portion of total cases, approximately 4% in the United States [21]; thus, sporadic cases are much more common. Incidence worldwide is presumably vastly under-reported [22] due to a combination of factors, including heavy reliance on a serogroup 1 *L. pneumophila*-specific urine antigen test for medical diagnosis [23]. Incidence has been rising consistently since the discovery of the disease [24], with a nearly 3.5-fold increase between 2000 and 2011 in the United States [23], leading to legionellosis being considered an increasingly important disease from a public health standpoint.

Legionnaires' disease is an atypical pneumonia with a 2–14 day incubation period [17] during which *Legionella* infect lung tissue, primarily alveolar macrophages [8]. Between 2009 and 2013, a mortality rate of 9% was reported in the United States, although this rate can be highly variable [23]. Conversely, Pontiac fever presents as a febrile, self-limiting disease with a 24–48 h incubation period [17]. Representing 0.5% of total legionellosis cases in the United States [21], Pontiac fever is less commonly associated with reported outbreaks than is Legionnaires' disease. Because its symptoms are similar to those of influenza infections and due to poor consensus on a clinical definition for the disease [7], Pontiac fever is rarely reported and poorly understood [25], although evidence suggests it to be relatively common [7]. At least some cases may be caused by an allergic reaction to a toxin produced by *Legionella* rather than infection [26], but data supporting this notion are limited. Extrapulmonary forms of legionellosis occur, such as endocarditis [27], osteomyelitis [28], and skin infections [29], although these are typically seen in patients who are immunocompromised, a major risk factor for the disease [23]. While more common species and strains of *Legionella* are capable of causing these atypical forms of legionellosis [30], less pathogenic strains are often implicated [31,32] due to lowered immune response often seen in patients with these infections. Old age [23], smoking [33], diabetes [34], and COPD [23] are also among the most common comorbidities positively associated with legionellosis. Despite these risk factors, relatively healthy individuals are also commonly infected [35], making the term “opportunistic pathogen” somewhat of a misnomer for *Legionella*.

As humans are an incidental host for *Legionella*, legionellosis is not typically considered transmissible from person-to-person [17]; however, a single presumptive case has shown evidence of lateral transmission [36]. With an ideal growth range between 28 and 40 °C [37], heated water systems are thought to serve as the primary source for *Legionella* occurrence and transmission, although a wide variety of sources have been linked to legionellosis [38]. Many engineered water systems tend to have physical conditions conducive to *Legionella* [39], biofilms they associate with [40], and eukaryotic hosts they replicate within [41], all factors commonly found and amplified in premise plumbing (i.e., that found in buildings such as homes, schools, and hospitals) [42].

Transmission from contaminated environmental sources occurs via inhalation of aerosols [43] or aspiration of fluid [44]. Inhalation occurs when *Legionella* cells or particles containing them enter the respiratory tract, with aerosol sizes of <10 μm needed for alveolar deposition [45]. Aspiration occurs when physiological barriers, such as the gag reflex, fail to prevent fluid from the mouth or stomach entering the lungs and is most commonly seen in recumbent patients or those with throat obstructions [46]. While commonly seen in typical pneumonias [47], this form of transmission is poorly documented for legionellosis. Transmission of rare forms of legionellosis, most commonly occurring in immunocompromised patients, can occur via direct skin contact [48] or exposure during medical operations [49], along with systemic infection transmission.

Q2) Are there any climatic, socioeconomic, or regional trends associated with legionellosis?

Legionellosis follows a distinct seasonal pattern differing from that of other forms of pneumonia [50], but mirroring that of many water-borne diseases with a peak incidence in the summer [17]. There is also an increase in incidence of cases caused by one species, *L. longbeachae,* in the spring in certain regions [51]. While warmer weather has been shown to be associated with increased presence of *Legionella* in natural water systems [52], legionellosis incidence does not appear to follow climate zone trends in the United States [23]. This is likely due to capability for engineered water systems to provide ideal conditions for the growth of *Legionella* year-round [53]. Despite this, evidence has suggested specific meteorological conditions influence legionellosis transmission rates. Rain events [54], high humidity [55], and low pressure [56] have been repeatedly shown to be positively associated with the number of reported cases, although there have been some dissenting results, potentially due to regional climate differences. Results demonstrating a larger impact from local watershed hydrology than from weather on legionellosis risk suggest that geographic influence on legionellosis extends beyond supporting increased aerosol transmission, at least in certain circumstances [57]; the concentration of *Legionella* in the source is also important when considering these factors.

There may be links between demographic or socioeconomic factors and legionellosis, but there is a paucity of data on this topic. Multiple state public health agencies in the United States have shown people of African descent to represent a disproportionately high number of legionellosis cases [58,59]. In a nationwide examination of public health data by the Centers for Disease Control (CDC), incidence among this group was substantially higher than for whites [23]. Additionally, residents of low-income neighborhoods in the state of New York have also been shown to have increased odds of contracting legionellosis [59]. Further studies will be necessary to determine if these two risk groups are due to medical (comorbidities, limited health care), environmental (low-quality drinking water, premise plumbing deficiencies), or social (smoking rates, high-risk jobs) reasons.

Cases of legionellosis in occupational settings have been documented [60], and workers in certain professions with exposure to aerosols may be at higher risk for legionellosis. For example, workers at wastewater treatment plants may be exposed to exceptionally high levels of *Legionella* aerosols [61–63]. Studies from several countries have also provided evidence suggesting professional drivers to be at increased risk [59,64,65]. Further examination of risk for other work-related exposure may reveal increased legionellosis rates associated with other professions having feasible exposure pathways, such as agricultural workers.

Reported legionellosis incidence and patterns of disease can vary greatly based on political boundaries, potentially due to factors other than climate and geography. An example of this is seen in the United States, where the state of New York reports approximately 50% of the cases of legionellosis in the entire country, even though it makes up only 6% of the population; New York's reported incidence is 10 times higher than California's [23]. The difference appears to be attributable to a state public health system with higher awareness and response to Legionnaires' disease not seen in other parts of the United States, a notable example being recent ordinances mandating cooling tower monitoring and treatment, inexorably placing legionellosis prominently in the public eye [66]. Regional variation in legionellosis rates caused by species other than *L. pneumophila*, mainly *L. longbeachae*, are not as readily explained. Responsible for less than 2% of reported cases in the United States [23], *L. longbeachae* is more frequently seen in other nations, namely Australia and New Zealand [22,67], where 42% and 85% of cases, respectively, are attributed to this species. While further environmental studies are necessary to determine the occurrence of *Legionella* in various sources in regions across the world, geographical differences in public health awareness concerning various aspects of legionellosis, such as the potential for soil-based transmission, may be responsible for these regional trends.

Q3) Is Legionella transmission more likely in certain types of built environments?

Based on epidemiological data, one is more likely to contract legionellosis in public places, such as hospitals, hotels, and nursing homes, than in private ones [1,25], but transmission can also occur in homes [68]. However, these observations may be biased because legionellosis outbreaks are more easily detected in public places than private residences. An updated, large-scale epidemiological study comparing legionellosis incidence in public buildings vs. private residences would be informative. The first recognized outbreak of legionellosis occurred in Philadelphia in 1976, where 182 attendees of an American Legion convention at a hotel were infected with *Legionella*, and 29 of those infected (16%) died [2,3]. Initially the source of *Legionella* was not found, but investigators later hypothesized that the bacteria came from the hotel's air conditioning system; however, to our knowledge there has not been any published study confirming the source. Two years later another legionellosis outbreak occurred in Memphis, Tennessee, this time at a hospital [69]. This outbreak was traced back to the hospital's air conditioning cooling tower, where *L. pneumophila* was recovered in water samples.

As most transmission of *Legionella* appears to involve aerosolization of bacteria from water into air, transmission is more likely in built environments that contain aerosol-generating features. Bauer et al. [70] showed that *Legionella* in shower aerosols increased the risk of Pontiac fever in residents in retirement homes. In 1999, in one of the largest reported outbreaks, ~200 attendees at a flower show in the Netherlands contracted Legionnaires' disease [34]. The source of *L. pneumophila* was eventually traced back to whirlpool spas that were in the exhibition halls. This was not the only case of whirlpool spas causing legionellosis. Another outbreak in Europe occurred in 1999 during the annual fair in Belgium, where 93 people were suspected to have contracted legionellosis and 41 cases were confirmed [71]. Although the source of *Legionella* was never verified, epidemiological data suggests that candidates included a whirlpool, steam iron, rainproof roof, fountain, and aquarium. In 1994, 50 cruise ship passengers were infected with *Legionella*, and hot tubs were thought to be the source of the bacteria [72]. Legionellosis outbreaks have also been associated with decorative fountains [73,74]. In 2005, 18 cases of community-acquired Legionnaires' disease were traced back to a decorative fountain at a restaurant in South Dakota [73]. As soon as the fountain was removed, disease transmission stopped. Similarly, eight patients with confirmed cases of Legionnaires' disease in 2010 in Wisconsin were exposed to bacteria from a contaminated decorative fountain in a hospital building [74].

Although most reported legionellosis outbreaks occur in large buildings, transmission can occur in homes. One explanation for why large buildings appear to be more likely places for legionellosis transmission is that large outbreaks seem to stem from exposure of many people to a single source, while disease transmission in homes appears to go unreported frequently. Additionally, it is possible many cases of Pontiac fever, which might not result in a visit to a health care provider, occur in homes; however, no studies to date have examined this issue in detail. In addition to potential *Legionella* exposure risk from showers, other aerosol-generating devices in the home, including faucets (discussed in detail in Q7), have also been shown to contain *Legionella* and pose a risk. Tyndall et al. [75,76] found *Legionella* in home humidifiers. Medical nebulizers have also been suggested as a potential *Legionella* source, and although they are typically used in hospitals, patients do bring them home regularly [77]. Future research and epidemiological data are needed to establish whether homes pose a more significant risk for legionellosis than do other buildings (see Q10).

Q4) What are the major sources of community-acquired legionellosis?

Nosocomial legionellosis, both sporadic and outbreak-related, has been relatively well studied [78], with a majority of cases linked to premise plumbing [79], cooling systems [80], or aerosol-generating medical equipment in hospitals [81]. Community-acquired legionellosis, responsible for 75% of reported cases [24], is much less understood, particularly for sporadic cases [82], although aerosolized tap water from heated engineered systems is presumed to be the major source of infection. Cooling towers are perhaps the most widely known source of legionellosis [83,84]. These devices can generate large volumes of aerosols from a heated water source that is frequently contaminated by *Legionella*, and their aerosols can be produced over extended periods of time and disseminated over long distances [85,86].

Epidemiological evidence is weaker for transmission within residences, despite the fact that *Legionella* can frequently be found in home tap water. *L. pneumophila* serogroup 1 has been detected in 47% of cold water taps across the United States [87]; hot water taps were not tested. While showers and faucets have the potential to serve as sources of transmission [88,89], data to quantify relative risk for transmission routes in homes are lacking. Showering is widely presumed to be the most likely source of aerosolized *Legionella* in homes, but modeling efforts have concluded that concentrations higher than those typically observed in residential premise plumbing [87] are needed for meaningful transmission via this route [45]. Other documented sources associated with high water concentrations and/or generation of large volumes of aerosols, such as humidifiers [75,76] and nebulizers [77] could have a high potential for community-acquired transmission; however, we contend that their relatively low frequency of use among the public precludes them from being among the most common sources (see Q5 for further discussion on these potential sources). Further research on legionellosis etiology outside of hospital settings may shed light on other unknown or poorly understood forms of transmission, as suggested by the authors of an epidemiological study in England and Wales linking 20% of sporadic community-acquired cases to motor vehicles with improperly filled windshield washer fluid reservoirs [65]. Wastewater treatment facilities are another source of *Legionella*. It is well known that bioaerosols are released from wastewater treatment plants [90,91], and multiple studies have shown that sewage and aeration ponds can contain very high concentrations of *Legionella* (greater than 10^9–10^{10} colony-forming units (CFU) L^{-1}) [61,92–94], although the concentration of *L. pneumophila* is probably much smaller.

Q5) What are other sources that may be overlooked?

Hines et al. [95] reviews in detail water sources that have been identified to contain *Legionella* and concentrations or emission factors from those sources. Major sources in the built environment include plumbing sources (e.g., showers, faucets, and toilets), cooling towers, respiratory devices (e.g., humidifiers, vaporizers, and nebulizers), swimming pools (including spas/hot tubs and whirlpools), steam-producing appliances, and ornamental fountains. As many outbreaks have been traced back to these sources, most of the previous research on *Legionella* has focused on them. However, it is possible that we are overlooking many other potential sources, such as natural water (e.g., lakes and rivers), windshield washer fluid, home air conditioning units, and other water-containing fixtures. In fact, *Legionella* has been found in many of these "overlooked" sources; however, to our knowledge none of them have been identified as the source of any major disease outbreak.

Although cooling towers have been identified as a major source of *Legionella* in multiple outbreaks (see Q3), home heating, ventilation, and cooling (HVAC) systems have remained unexplored [96]. HVAC systems, including ductwork, have been shown to be a significant source of bioaerosol emissions, especially bacteria and fungi [97–99]. Bacterial concentrations in excess of 4.7×10^4 CFU cm^{-2} have been recovered from heat exchangers in HVAC systems. Additionally, HVAC filters have been suggested to favor the growth of bacteria when relative humidity is high [100]. Considering that (1) legionellosis is more common in the summer months (see Q2), when HVAC systems are heavily used; (2) *Legionella* grows best at temperatures between 20 and 45 °C [101]; (3) home HVAC systems are typically located in attics, where the temperature may fall in *Legionella*'s preferred range during the summer months; and (4) HVAC systems produce an abundance of water, much of which sits in drip pans for long periods, it is plausible that HVAC systems could be a source of legionellosis. It is possible that many of these cases of legionellosis would be overlooked, as they would not be associated with a major outbreak.

Other sources associated with the built environment have also been identified. Schwake et al. [102] detected *Legionella* in windshield washer fluid samples collected from school buses. This finding provides support for epidemiological studies showing that driving for a profession is associated with enhanced risk of legionellosis [33,103]. Researchers hypothesize that aerosols are generated when using windshield washer fluid and that some of these aerosols enter the vehicle cabin.

As legionellosis has been shown to be caused by bacteria that have traveled long distances in the atmosphere [104–106], it would be negligent to overlook potential natural environmental sources of *Legionella* aerosols that could be transported into built environments. In 2005, a community-acquired outbreak of Legionnaires' disease occurred in Spain [85], and the source of *Legionella* was traced back to cooling towers. In 22% of the cases, exposure to bacteria occurred between 1800 and 3400 m from the source, supporting the idea of long-distance transport. Recently, researchers showed that when a rain droplet hits soil, 0.01% of the bacteria on the soil surface are emitted into air as a bioaerosol [107]. *Legionella* has been shown to persist in soil [108–110], and it is possible that the bacteria could be aerosolized either through rainfall or wind-induced suspension. Although some of the bacteria in the soil may come from industrial waste or cooling towers, Rowbotham [111] suggested that amoebae in soil might enhance the growth of *Legionella*. Other potential sources include rainwater puddles, which have been found to harbor viable *L. pneumophila* [112], and rooftop rainwater cisterns [113–116]. *Legionella* appears to be part of the natural aquatic microbiome, as it has been found in oceans, lakes, and rivers [117,118].

The possibility of *Legionella* bioaerosols being emitted from outdoor sources, transported long distances, and entering the built environment is not unrealistic, especially considering the high penetration efficiency of outdoor bioaerosols into indoor environments [119]. However, in order to assess the potential for long-distance transport, it is important to understand how well *Legionella* survives in aerosols (see Q8). There are ample opportunities for future research examining these potentially overlooked sources of legionellosis (see Q10).

Q6) What are typical concentrations of Legionella in water sources and what factors contribute to growth in water systems?

Concentrations of *Legionella* in water sources can vary wildly depending on the source, ambient conditions, and specific events (Table 1). Because of naturally occurring fluctuations in numbers [120], impact of various control methods [121], and sporadic changes in bulk water concentrations due to periodic biofilm detachment [122], determining a typical level of *Legionella* for a given environment is difficult. This issue is exacerbated by a lack of in-depth monitoring studies implementing multiple quantification methods. The two most common approaches to enumerating *Legionella*, quantitative polymerase chain reaction (qPCR) and cultivation, are inherently difficult to compare and can produce either similar or radically different results from identical samples [123–125]. Thus, careful interpretation is required to properly understand any *Legionella* occurrence data. Concentration data have been collected at length for many environmental sources frequently contaminated with *Legionella,* although more research designed to assess the aggregated data will be needed to determine truly "typical" concentrations for various sources.

Legionella are able to adapt to a wide variety of environments, surviving long periods in harsh, oligotrophic conditions such as those found in chlorinated drinking water systems [126–128], only to proliferate in response to favorable stimuli. Favorable levels of heat [101,129], mature biofilm accumulation [130,131], and presence of eukaryotic hosts [132,133] are the most frequently observed factors responsible for proliferation. Source-specific conditions, such as low disinfectant residual [134] and stagnant water [135], both frequently found within premise plumbing, have also been shown to be conducive to *Legionella* growth. Many water quality parameters frequently associated with poor microbial quality, such as organic carbon [136] and turbidity [137], have little or sporadic impact on *Legionella*, while the presence of certain metals such as iron, copper, manganese, and nickel have shown either positive or negative effects in different systems.

Specific events occurring in *Legionella* sources may facilitate rapid population "blooms," aiding in transmission and increased risk of legionellosis. Water system deficiencies, such as insufficient disinfection, improper maintenance, and main breaks, are frequently implicated in legionellosis clusters and have been noted

Table 1
Concentrations of *Legionella* found in common water sources.

Source	*Legionella* concentration				Reference
	Total Bacteria (genome units L^{-1})		Culturable Bacteria (CFU L^{-1})		
	Minimum	Maximum	Minimum	Maximum	
Cooling towers	NA	NA	1.2×10^6	1.0×10^7	[142]
	NA	NA	1.0×10^5	1.2×10^9	[143]
	6.3×10^2	2.5×10^6	NA	NA	[120]
Drinking water	NA	NA	1.0×10^2	1.3×10^4	[144]
	NA	NA	3.0×10^6	9.0×10^8	[142]
	NA	NA	5.0×10^3	4.0×10^4	[145]
	NA	NA	4.0×10^1	9.5×10^5	[146]
	1.2×10^4	1.1×10^8	NA	NA	[139]
	1.0×10^4	2.3×10^6	NA	NA	[147]
	3.9×10^3	1.0×10^4	5.2×10^3	7.3×10^3	[124]
	8.1×10^2	3.2×10^5	7.9×10^1	1.5×10^4	[148]
Drinking water distribution system	3.0×10^3	3.2×10^8	NA	NA	[148]
	8.5×10^1 (mean)	5.9×10^3	NA	NA	[133]
	1.3×10^2	5.7×10^3	<50 (LOD)	NA	[149]
Decorative fountains	NA	NA	NA	3.0×10^6	[73]
	NA	NA	3.1×10^3	4.1×10^4	[74]
Hot tubs	1.0×10^3	6.1×10^7	2.5×10^2	3.5×10^5	[150]

in all 23 outbreaks in the United States between 2000 and 2014 investigated by the CDC, with inadequate disinfectant found in 16 of the outbreaks [138]. Similarly, improper maintenance, such as lack of regular cleaning of cooling towers, has been implicated in numerous outbreaks across the world [84]. Evidence from a recent pair of legionellosis clusters in Flint, Michigan suggests rapid disinfectant decay and damage resulting from corrosive source water throughout a municipal drinking water distribution system played a role [139]. In light of decaying water infrastructure in the United States and other nations, there may be potential for increased incidence of legionellosis [140,141].

Q7) What are the infectious doses of Legionella needed to cause disease and what do we know about the transfer of Legionella from water to air?

Early infectivity assays for *Legionella* demonstrated lethality in guinea pigs that were exposed to approximately 1,000–10,000 aerosolized cells [151], while more recent quantitative microbial risk assessment predicts an approximately 1 in 10,000 risk from one cell deposited in the lungs for humans [152]. These values, however, may not be reflective of real-world conditions and are highly dependent on many factors such as strain/species, cell state, aerosol size (which affects deposition efficiency in the respiratory tract), and host immunity. The wide range of risk demonstrated in various studies reflects not only the complex nature of legionellosis, but also that of *Legionella* themselves. These factors, combined with the aforementioned difficulty in comparing different quantification assays, make the infectious dose difficult to determine accurately. In a recent attempt to predict risk while showering, Schoen and Ashbolt [45] developed an exposure model assuming a 15 min shower. It was estimated that *Legionella* concentrations of 3.5×10^6–3.5×10^8 CFU L^{-1} in water, 3.5×10^1–3.5×10^3 CFU m^{-3} in air, or 7.8×10^5–7.8×10^8 CFU cm^{-2} in biofilm would be required to likely cause Legionnaires' disease.

Showers, bathtubs, and faucets are thought to be an important source for legionellosis. Crimi et al. [153] conducted a comprehensive study correlating *Legionella* contamination in water and aerosols from bathtubs in a hospital building. Although L. *pneumophila* was aerosolized from contaminated water, it became so diluted in air that it was undetectable at a distance of 1 m or more from the source. Bacteria concentrations in water samples ranged from 0 to 3.2×10^4 CFU L^{-1}. *Legionella* concentrations in air samples taken 5 cm, 50 cm, 100 cm, and 200 cm from the water tap were 0–55, 0–51, 0–5, and 0 CFU m^{-3}, respectively. Surprisingly, there was not a direct correlation between bacteria concentrations in water and air (i.e., a higher concentration in water was not always predictive of a higher concentration in air). Most importantly from a public health standpoint, *L. pneumophila* concentrations detected in the water samples were considered low risk for causing legionellosis, except for exposure within 5 cm of the tap, based on QMRA models [45]. Bollin et al. [89] examined aerosols generated by showerheads and hot-water faucets for *L. pneumophila* and found very low concentrations of bacteria in air collected within 1 m from the plumbing source (2.3–11.6 CFU m^{-3}). Ninety percent of *L. pneumophila* recovered were in aerosols between 1 and 5 μm in diameter, sizes capable of alveolar deposition [154]. Similar to other studies, when Dennis et al. [155] examined *L. pneumophila* aerosol generation by plumbing in bathrooms, they found very low concentrations (0.27–0.44 CFU m^{-3}) of bacteria in the air during the first 15 min of showering and no airborne *Legionella* during 15 and 30 min of showering, even though concentrations of *L. pneumophila* in the water were approximately 10^3 CFU L^{-3}. At a breathing rate of 12 L min^{-1}, over 4 h of showering would be required to inhale just one "viable" *Legionella* cell, although the researchers likely meant to use the term "culturable" since viable but non-culturable (VBNC) cells were not distinguished in their study. Perkins et al. [156] studied the total microbial load, not just *Legionella,* in shower water and shower aerosols in a hospital and found concentrations of 2.2×10^7 cells L^{-1} and 3.4×10^4 cells m^{-3}, respectively. Additionally, the researchers compared microbial loads in air with and without a shower running and concluded that any change in airborne microbial load due to showering was not distinguishable from background levels. Over 30 years ago, Muder et al. [1] concluded, *"Given the ubiquitousness of L. pneumophila in water distribution systems, it is obvious that mere isolation of the organism from showers should not necessarily be linked to causation. Increased exposure to showers has never been rigorously demonstrated for cases as opposed to controls, and, in our experience, many patients acquiring the disease were never exposed to showers."* This conclusion should be viewed with caution, as it is possible that VBNC *Legionella* are aerosolized from these sources. Additionally, most of these studies were conducted 20–30 years ago, and research techniques have greatly improved since then. Future studies are needed to determine the risk of legionellosis from showers (see Q10).

Cooling towers are considered another major source for legionellosis outbreaks [1,69,157]; however, to our knowledge there are few studies correlating concentrations of *Legionella* in cooling tower water with those in air. Ishimatsu et al. [158] found *L. pneumophila* concentrations in a contaminated cooling tower to be 1.2×10^6 CFU L^{-1}, while bacteria concentrations in outdoor air around the cooling tower were 90 CFU m^{-3}. One of the major challenges in trying to understand the relationship between waterborne and airborne concentrations of *Legionella* is that researchers are often unable to collect water and air samples simultaneously when an outbreak is occurring (see Q10). Another issue inherent to measurements of *Legionella* in water systems is that feasible sampling regimes may not accurately reflect the source due to spatial variation in the microbiome and the small fraction of the total system volume being analyzed. Typical field sampling procedures for *Legionella* could, thus, be considered analogous to collecting a teaspoon of water from the middle of a lake and trying to draw conclusions about the microbial community at the shore.

Although plumbing and cooling towers are typically considered the major sources of *Legionella*, airborne concentrations generated by other water sources have also been examined. Schwake et al. [102] detected *Legionella* in 84% of windshield washer fluid samples collected in school buses at concentrations as high as 8.1×10^7 CFU L^{-1}. *Legionella* was aerosolized from the windshield washer fluid, producing airborne concentrations next to the buses of 90–135 CFU m^{-3} in three samples; the bacteria were not detectable in two other air samples. Some of the highest airborne concentrations of *Legionella* reported in the literature, up to 3.3×10^3 CFU m^{-3}, were measured directly above aeration ponds at biological treatment plants [159]. In fact, viable *Legionella* was recovered 200 m downwind of an aeration pond, indicating that long-distance transport can occur. For comparison, *Legionella* concentrations greater than 10^9–10^{10} CFU L^{-1} in aeration ponds have been reported [61,92,160]. Further, epidemiological studies have suggested *Legionella* can be dispersed greater than 10 km from wastewater treatment plants [105]. In a study of the risk of legionellosis in gardeners, *Legionella* was found in 22.2% and 25.0% of tap water used for watering plants in samples collected outdoors and inside greenhouses, respectively [161], but no *Legionella* was recovered in air samples. Finding *Legionella* in natural aquatic habits (see Q5) is important since bacteria could be aerosolized from them though bubble-bursting and breaking-wave mechanisms, and could then access the turbulent boundary layer for long-distance transport [162,163].

Although progress has been made in developing a complete understanding of legionellosis transmission, there still remain many knowledge gaps when it comes to understanding water-to-air transmission of *Legionella* and which water sources emit the highest concentration of airborne bacteria. We discuss future research needs in Q10.

Q8) How well does Legionella survive in aerosols?

Legionnaires' disease develops when bacteria are deposited into the alveoli, presumably most commonly through inhalation of contaminated aerosols; however, aspiration of contaminated water has been suggested as another transmission route [44,164]. A key piece to the puzzle regarding legionellosis transmission that has received limited attention is a complete understanding of how well *Legionella* survives once aerosolized and what factors affect viability. Some studies have shown that *Legionella* in aerosols can survive for several hours and that viability depends on humidity and bacterial strain [165–167].

Berendt [165] found a direct relationship between relative humidity and survival of airborne *L. pneumophila*. The times for *L. pneumophila* viability to decrease by a factor of two were 15.6 min, 10.3 min, and 3.2 min at 80%, 50%, and 30% relative humidity, respectively, suggesting that the bacteria are most stable at higher humidity. In contrast, Hambleton et al. [166] showed that *L. pneumophila* survived best at 65% relative humidity and worst at 90% and 30% relative humidity. Additionally, Hambleton et al. [166] criticized the use of only three relative humidity values when studying viability due to the presence of specific zones of instability. The researchers aimed to validate this statement by testing viability of aerosols after 15 min at seen relative humidities: 90%, 80%, 70%, 65%, 55%, 40%, and 30%. Although survival was best at 65%, it was significantly lower at 55% viability compared to either drier or more humid air. Contrary to the other studies, Dennis and Lee [167] found the best survival at 90% relative humidity, intermediate survival at 30% relative humidity, and poor survival at 60% relative humidity. Making a direct comparison between studies is difficult as the aerosolized bacteria were suspended in different media, and experimental methodology differed in each case. Tang [168] has suggested that just a 5–10% difference in relative humidity can define the best vs. worst conditions for survival of aerosolized *Legionella*. From these studies, it is evident that *Legionella* viability is not as trivial as “the lower the humidity, the fewer bacteria survive.” A complete understanding of the mechanistic effect of relative humidity on *Legionella* survival is necessary, particularly since there can be large fluctuations in relative humidity in a built environment throughout the year.

Although multiple studies have examined the effect of temperature on *Legionella* growth and survival in water systems [37,169–171], to our knowledge no studies have examined the effect of air temperature on survival of aerosolized *Legionella* in the built environment. Some studies have examined climatic trends associated with legionellosis (see Q2); however, it is not known if these trends are more directly linked to bacteria survival/transmission or seasonal practices (such as using air conditioning/cooling towers more), or a combination of the two. The effect of temperature on airborne *Legionella* survival may be less relevant because indoor air temperature in the built environment tends not to vary much, compared to typical fluctuations in water temperature or relative humidity.

While relative humidity and temperature are thought to be major factors affecting bioaerosol viability, other variables should not be overlooked. Muraca et al. [172] showed a 5-log reduction in the number of viable bacteria in water when exposed to ultraviolet light for just 20 min. Tong and Lighthart [173] showed that solar radiation had a lethal effect on airborne bacteria collected outdoors. Presumably, solar radiation has the greatest impact on *Legionella* originating from outdoor sources (e.g., wastewater treatment plants, windshield wiper fluid). Understanding *Legionella*'s tolerance for solar radiation is important to predict the relevance of long-distance transport of *Legionella* in the atmosphere.

In order for *Legionella* to be inhaled and cause disease once aerosolized, it must remain airborne. A driving factor that determines how long an aerosol remains airborne is its size. The larger the aerosol, the higher the settling velocity and the more rapidly it will be removed from air [174]. Zhou et al. [175] reported that showers using hot water generated aerosols with a mean diameter of 6.3–7.5 μm. Interestingly, the aerosol size was independent of the water flow rate, but when cold water was used, aerosols were about half the size. Future research is needed to examine size-resolved emissions for different sources of *Legionella* (see Q10).

Q9) What engineering practices can we implement to reduce disease transmission?

There are many engineering practices that have the potential to reduce legionellosis transmission in the built environment. However, before beginning to explore engineering controls, we must first have a more complete understanding of how *Legionella* is transferred from water to air. Engineering practices should focus on the major sources of legionellosis in built environments to make them less hospitable for *Legionella* growth and emission into the air (see Q4 and Q5).

The application of physical and chemical control methods to prevent *Legionella* growth and persistence in plumbing systems has been well reviewed [176,177]. Darelid et al. [178] conducted a 10-year legionellosis surveillance program and concluded that keeping the circulating hot water temperature above 55 °C would limit bacteria growth and thus control disease outbreaks. In fact, hot water temperatures are required to be above 60 °C in some countries [179], although organizations in the United States recommend a temperature of 51 °C, which is near the favorable growth temperature for *Legionella* [101,180]. Both water age [181,182] and distance from the water heater [101] are positively correlated with *Legionella* growth, so reducing these could be a control strategy.

Disinfectant type and pipe material might affect *Legionella* persistence, with monochloramine showing the potential to prevent legionellosis [182,183]. Triassi et al. [184] found that zinc levels below 100 μg L^{-1} and copper levels above 50 μg L^{-1} inhibited *Legionella* growth in plumbing systems. On the contrary, Rakic et al. [185] showed that zinc had no effect on *L. pneumophila* presence, while iron and manganese were positively correlated with bacteria presence. Researchers have also suggested that *Legionella* might preferentially colonize biofilms and detach more readily on copper surfaces [186]. The conflicting results indicate that more variables and complex interactions between them may be at play, and future research is needed to discern these. However, it is unclear whether these metal levels will provide long-term protection against *Legionella* growth since there is evidence that copper levels are positivity correlated with disinfectant loss [187,188].

UV light and ozone are other engineering controls to inhibit *Legionella* in plumbing systems [172,189]. Muraca et al. [172] showed that both UV light and heating water above 60 °C caused a 5-log reduction in *Legionella* within an hour; however, ozone and chlorine required a 5-h exposure time to achieve the same level of reduction. Further complicating treatment options is the movement towards “green” buildings. Future research is needed to determine how to balance *Legionella* control with energy savings and environmental concerns.

Since *Legionella* can be transmitted via aerosols, we should begin to examine ways to disinfect air and make it less hospitable for the bacteria. Unfortunately, there has been limited research examining disinfection strategies of *Legionella* in air, as the focus has been on preventing bacteria growth in plumbing systems. Thus, aerosol-focused remediation approaches should be further developed and validated. Disinfection strategies for cooling towers and air ducts should be examined due to numerous cases of legionellosis being traced back to these sources. Manufacturers of cooling towers recommend treatment and cleaning protocols, but their efficacy has been questioned and needs to be further studied for *Legionella* control [190]. Another potential engineering control that has shown to be effective for other respiratory pathogens is the incorporation of UV germicidal irradiation (UVGI) into indoor air systems for disinfection [191,192]. Using UV or photocatalytic treatments for disinfection of airborne *Legionella* has shown some promise [193,194]; however, fungi might protect bacteria from these types of treatment [195]. In addition to disinfecting the air, it is also important for cooling systems to be regularly cleaned to inhibit biofilm growth [196,197].

Other control strategies to reduce disease transmission might be as simple as regularly cleaning potential sources of *Legionella* (e.g., hot tubs, water storage tanks, decorative fountains). If aeration ponds at wastewater treatment facilities are found to be a significant source for legionellosis outbreaks, covering them or changing the aeration system can reduce the number of bioaerosols (and potentially *Legionella*) released [91]. Air diffuser aerators produce about an order of magnitude less bioaerosols compared to horizontal and surface turbines.

Recently, scientists and engineers have begun to shift from a viewpoint of trying to kill all microorganisms in the built environment instead to trying to harness the beneficial microbes and use a probiotic approach to make our buildings healthier [198,199]. Some *Bacillus* have shown the ability to inhibit the growth of *L. pneumophila* [200]. In cooling towers, the use of *B. subtilis* decreased the concentration of *L. pneumophila* from 53,000 CFU L^{-1} to less than 1,000 CFU L^{-1} in three weeks. Before practitioners jump to a probiotic approach to limit legionellosis outbreaks, more work is needed to determine the efficacy and safety of this strategy.

Q10) What future research is needed?

Although we have made significant strides in developing a complete understanding of legionellosis and *Legionella* transmission, many questions and misconceptions remain. Research is needed to fully understand the water-air-human transmission pathway of *Legionella* using controlled laboratory studies. Additionally, epidemiologists and scientists need to examine whether overlooked sources, discussed in Q5, are a potential risk for disease transmission.

Conventional wisdom is that showers and faucets are a major source for *Legionella* in the built environment due to the large volume of water being sprayed/aerosolized and daily exposure to them. However, evidence is circumstantial and largely based on epidemiological data. To our knowledge, no studies have tracked *Legionella* from shower water to aerosols and shown concentrations of culturable bacteria at high enough concentrations to produce favorable odds of disease (see Q7). It is challenging to collect water and aerosol samples on short notice while a legionellosis outbreak is occurring, and most field studies have been more of a fishing expedition for *Legionella*. If showers are to be considered a source with significant risk for causing legionellosis in the built environment, then there must be a controlled study in which shower water (or a biofilm) is inoculated with a known amount of *Legionella* and the concentration of *Legionella* in shower aerosols is measured. Admittedly, there are many challenges to this type of study, especially when it comes to institutional biosafety concerns.

There are multiple methods for *Legionella* detection and quantification, thus, we need a better understanding of their limitations and how they relate to one another. Researchers have suggested that direct comparisons between *Legionella* detection by qPCR and culture are difficult to interpret [123] and, thus, it is important to simultaneously determine the total concentration of *Legionella* bacteria through qPCR (viable and non-viable), culturable *Legionella* by culturing techniques, and VBNC *Legionella* through epifluorescence microscopy. A primary benefit of advancing *Legionella* detection methodology would be improving our understanding of the role that VBNC cells play in legionellosis transmission. Alleron et al. [201] showed that VBNC *L. pneumophila* cells still produce virulence proteins. Additionally, research has shown that VBNC cells can rapidly shift to other *Legionella* cell states in response to the correct stimuli, and in some cases might be more virulent, either due to increased resistance to stress or to greater infectivity [10,202,203]. However, Steinert et al. [204] showed that dormant *Legionella* is not reactivated in animals. We need a more complete understanding of how the physiology of *Legionella* regarding cell forms and differential gene expression affect disease transmission.

This topic is, potentially, even more relevant for aerosolized *Legionella*, yet there is a severe paucity of data in this particular research area. Similarly, research on *Legionella* species and strains other than *L. pneumophila* serogroup 1 is warranted, particularly for uncommon forms of legionellosis transmission and Pontiac fever. Differences in *Legionella* strains and cell forms used in previous studies could be another explanation for differences seen between studies that examined the effect of environmental conditions on survival and occurrence. Gaining a better understanding of how these forms differ will not only provide guidance for future research but will also provide additional context for past results.

It is evident that we still do not have a clear picture of how well *Legionella* survives in aerosolized form (see Q8). Studies have produced conflicting evidence about how relative humidity affects survival and to our knowledge no data exist examining the effect of temperature on aerosolized *Legionella*. Many of the studies examining the impact of relative humidity are more than three decades old and research techniques have significantly improved; therefore, these studies should be revisited. Additionally, it is difficult to understand the role of relative humidity on *Legionella* survival when only three humidity values are studied; we recommended that future research include a larger set of humidity values. We additionally need studies that examine the effect of solar radiation on *Legionella* survival. UV disinfection of airborne bacteria is not an independent factor, as UV disinfection can be affected by relative humidity [193,205]. Although relative humidity is likely to be the most important environmental factor affecting *Legionella* survival, for completeness, temperature and solar radiation also merit attention, as results could lead to various engineering controls to prevent legionellosis outbreaks. Finally, we need a more complete understanding of *Legionella* survival dynamics within aerosols although as discussed earlier we first need to understand if we should focus on VBNC or culturable *Legionella* [125].

Other recommended research includes studying the aerosol size and number emissions from different sources of *Legionella*. Aerosol size can have a cascading effect on everything from how long bacteria remain airborne to where they deposit in the respiratory system. It might be possible to engineer our built environment to create aerosols of sizes that are less favorable for legionellosis transmission (e.g., using low vs. high flow faucets). Additionally, further exploration of *Legionella* sources that have been overlooked (discussed in Q5) is needed to determine whether they pose a significant risk for legionellosis. If so, strategies to mitigate disease transmission from these sources should be developed. Legionellosis outbreaks are associated with summer months and the cooling season, and it is possible that home HVAC systems are contributing to disease outbreaks in addition to cooling towers; however, few studies have inspected *Legionella* in home HVAC systems. All of the future research needs described herein are intricately linked, making it difficult to prioritize specific topics.

2. Conclusion

Legionellosis is a serious disease responsible for many deaths since it was first discovered more than 40 years ago. Although we have made progress in understanding *Legionella* transmission, major questions and knowledge gaps remain. Some of the most important aspects of the disease's etiology revolve around water-to-air transmission and the number of viable bacteria aerosolized from different sources. Additionally, VBNC *Legionella* might be playing an underappreciated role in legionellosis due to this pathogen's pleomorphic nature. By addressing some of the unanswered questions, it is possible that we might identify simple interventions to reduce *Legionella* transmission. Ultimately, we aim to prevent legionellosis, and figuring out how to engineer our built environment to prevent disease transmission is a priority.

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