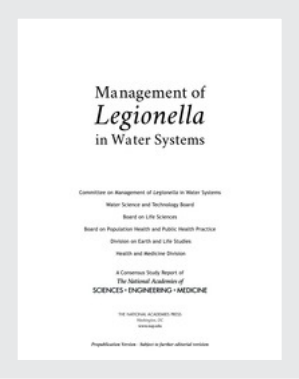

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Management of *Legionella* in Water Systems

Committee on Management of *Legionella* in Water Systems

Water Science and Technology Board

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Division on Earth and Life Studies

Health and Medicine Division

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Preface

Legionnaires' disease arrived on the scene in dramatic fashion during the 1976 Philadelphia outbreak that included 182 cases of pneumonia and 29 deaths. Almost 40 years later, major outbreaks at a community level (Flint, Michigan), in healthcare facilities (such as the Quincy, Illinois veterans home), and due to cooling towers (New York City) have again catapulted *Legionella* into national headlines. *Legionella* is now the number one cause of reported waterborne disease in the United States, transmitted through contaminated water that is aerosolized and exposing those nearby via inhalation into the respiratory tract.

The bacteria in the genus *Legionella* occur naturally in water but have optimal growth at warm temperatures. Wherever there are water and pipes eventually one can find *Legionella* including in many human-made building water systems. However, its exact niche and the factors influencing it to bloom are only now being elucidated. *L. pneumophila* is the species (among many) most often diagnosed as the cause of Legionnaires' disease. For every case associated with an outbreak there are nine more sporadic cases. Are these patients exposed in hospitals, from cooling towers, or within residences or commercial buildings such as hotels? Who is responsible for monitoring and controlling the bacteria and the disease? These are complex issues and despite major gains in knowledge about the bacteria, its ecology, its transmission, and Legionnaires' disease, there remains great uncertainty about how to control *Legionella* in water systems.

The National Academies of Science, Engineering, and Medicine were asked by the U.S. Centers for Disease Control and Prevention (CDC), the U.S. Department of Veterans Affairs (DVA), the U.S. Environmental Protection Agency (EPA), and the Alfred P. Sloan Foundation to address the state of the science with regard to *Legionella* including its ecology, disease diagnosis, amplification within water systems, quantification, prevention and control, policy and guidance, and all associated research needs.

This study was established under the auspices of the Water Science and Technology Board (WSTB) of the National Academies. The WSTB convened a Committee to address the management of *Legionella* in water systems that included 13 individuals with various backgrounds and expertise in *Legionella*. Over the course of two years, the Committee conducted a scientific literature review on the state of the science, covering the biology, taxonomy, and ecology of the bacteria; outbreaks and disease surveillance; environmental data from all types of building water systems; control methods; and rules and guidelines for addressing *Legionella* contamination. It conducted some original data analyses, and formulated conclusions and recommendations meant to improve management of *Legionella* contamination of water systems and consequently better control Legionnaires' disease in the United States.

The Committee recognizes that *Legionella* is only one of a number of pathogens found in water distribution systems and in building premise plumbing. Some of these other pathogens may be as serious as *Legionella*, such as *Mycobacterium avium* (and other non-tuberculous mycobacteria). The control of *Legionella* may have unintended consequences on these other organisms, as discussed briefly in Chapter 4. However, it was not the purpose of this Report to consider organisms beyond *Legionella*.

During its six committee meetings, the Committee heard from experts involved in characterizing, monitoring, and remediating *Legionella* as well as from those knowledgeable about *Legionella* control policies from Australia, Canada, and Europe. I would like to thank the following individuals for giving formal presentations to the Committee including Sam Posner, Laura Cooley, Jason Kunz, and Brian Raphael, CDC; Shantini Gamage, Gary Roselle, and Oleh Kowalskyj, DVA; Eric Burneson, EPA; Paula Olsiewski, Sloan Foundation; Janet Stout, Special Pathogens Lab; Tim Keane, *Legionella* Risk Management, Inc.; Jennifer Clancy, ESPRI; Christopher Crawford, New York City Department of Health and Mental Hygiene; Jessica Evans, NSF International; David Krause, Forensic Analytical Consulting Services; Alvin Bartels, the Netherlands; David Cunliffe, Australia; Martin Exner, Germany; John V. Lee, England; and Gary Klein, Gary Klein and Associates. The Committee also thanks the many individuals that spoke during open-mic sessions or submitted written comments to the Committee during the course of the study.

This Consensus Study Report was reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the National Academies of Sciences, Engineering, and Medicine in making each published report as sound as possible and to ensure that it meets the institutional standards for quality, objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process.

We thank the following individuals for their review of this report: Zia Bukhari, American Water; Anne Camper, Montana State University; Elizabeth Casman, Carnegie Mellon University; Jennifer Clancy, ESPRI; David Fisman, University of Toronto; Marian Heyman, Connecticut Department of Public Health; Sophie Jarraud, Lyon Medical School; Richard Miller, University of Louisville; Norman Pace, University of Colorado; and Caitlin Proctor, Purdue University.

Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations of this report nor did they see the final draft before its release. The review of this report was overseen by Rhodes Trussell, Trussell Technologies, Inc., and Glen Daigger, One Water Solutions, LLC. They were responsible for making certain that an independent examination of this report was carried out in accordance with the standards of the National Academies and that all review comments were carefully considered. Responsibility for the final content rests entirely with the authoring committee and the National Academies.

Joan B. Rose, *Chair*
Committee on Management of *Legionella* in Water System

Contents

Summary

Legionnaires' disease afflicts and kills more people in the United States than any other reportable waterborne disease. It is caused by bacteria of the *Legionella* genus, with the majority of diagnosed cases (from 80 to 90 percent) linked to *Legionella pneumophila*. Humans are primarily exposed to *Legionella* through inhalation of contaminated aerosols into the respiratory system. Patients infected with *Legionella* can develop pneumonia (classic Legionnaires' disease) or a milder flu-like condition called Pontiac fever; both conditions are referred to as legionellosis. Legionnaires' disease can be fatal, with between 3 and 33 percent of *Legionella* infections leading to death. Those at higher risk for developing Legionnaires' disease include the elderly, males, smokers, and the immunosuppressed. In the United States, the reported incidence of Legionnaires' disease increased more than five-fold from 2000 to 2017 (Figure S-1). Worldwide, the actual burden of Legionnaires' disease is generally acknowledged to be underreported, by as much as eight- to ten-fold.

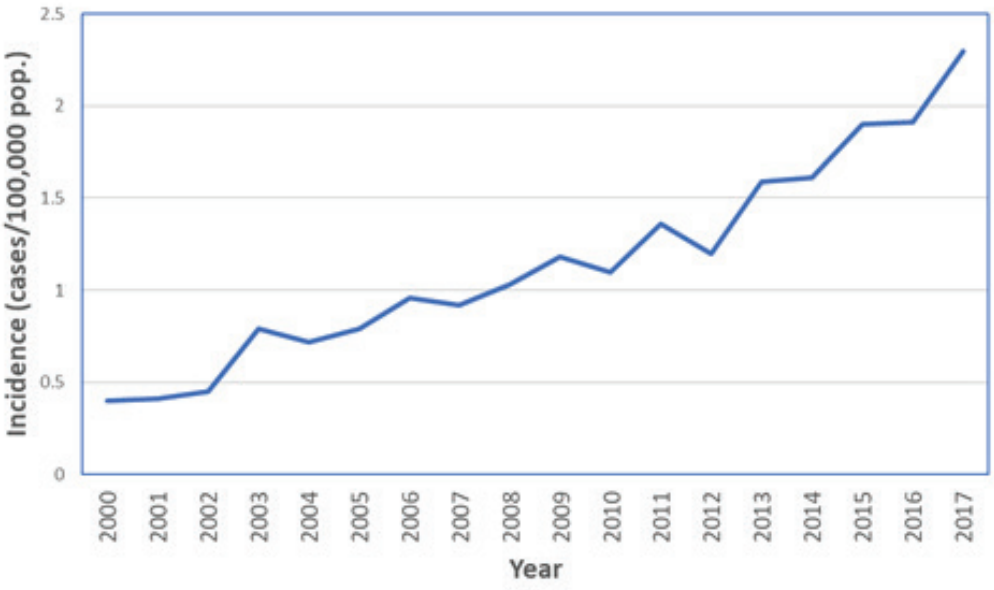

FIGURE S-1 Increasing incidence of legionellosis in the United States from 2000 to 2017. SOURCE: Adapted from Shaw et al. (2018) with 2016 and 2017 data from the National Notifiable Disease Surveillance System.

Legionella bacteria reside in many natural environments including rivers, lakes, and soils. They grow optimally inside protozoan hosts, such as free-living amoebae associated with microbial biofilms that coat wet surfaces. Hence, it is not surprising that these bacteria can be found in a variety of engineered systems that support biofilm growth, including drinking water distribution systems, building plumbing systems, faucets, showerheads, cooling towers, hot tubs, and fountains. These water systems are sometimes characterized by warm temperatures, stagnant water, excess nutrients due to pipe corrosion, and a lack of chemical disinfectants—conditions that promote the growth of biofilms, their associated protozoa, and consequently *Legionella*. When these built environments generate contaminated aerosols, humans in the vicinity can be exposed to *Legionella* via inhalation or aspiration. Most of our knowledge of Legionnaires' disease comes from investigations of disease outbreaks, instances where two or more people are infected at the same time by the same source. However, the vast majority of Legionnaires' disease cases (greater than 95 percent) are sporadic cases for which the primary exposure source is never identified.

In the 40 years since the discovery of *L. pneumophila*, much has been learned about its ecology, whereas less progress has been made in preventing Legionnaires' disease or understanding the ecology of different species of pathogenic *Legionella*. Methods for monitoring both the disease incidence and *L. pneumophila* in water samples have evolved considerably, for both culture-based and molecular techniques. Yet, treatment of water systems to reduce colonization by *Legionella* continues to be complicated by the microbe's complex ecology and the diversity of systems in which *Legionella* can thrive. Moreover, although the Safe Drinking Water Act has been effective in reducing disease rates of waterborne enteric organisms in the United States, it has had little impact on managing *Legionella* in water systems and buildings.

In late 2017, the National Academies of Sciences, Engineering, and Medicine convened an expert committee (the Committee on Management of *Legionella* in Water Systems) to review the state of the science with respect to *Legionella* contamination of water systems and issue a report that addresses the following:

- **Ecology and Diagnosis**: Describe the microbial ecology of water supplies (from the source to the tap and within built systems) as it relates to *Legionella*. What strains of *L. pneumophila* are of most concern and how can their diagnosis be improved (e.g., in terms of increased specificity, simplicity, and speed)?
- **Transmission via Water Systems:** What are the primary sources and routes of human exposure to *Legionella*? What features/characteristics of water systems make them more or less likely to support growth of *Legionella?*
- **Quantification:** Considering surveillance data, case studies of outbreaks, hospital data, other routine testing of water systems, what is known about the concentration of *Legionella* in water systems *and* the prevalence of Legionnaires' disease over the last 20 years? How uncertain are these estimates and what can be done to reduce this uncertainty? How can quantitative risk assessment be improved?
- **Prevention and Control:** What are the most effective strategies for preventing and controlling *Legionella* amplification in water systems? What are the best methods to prevent exposure to *Legionella,* especially in at-risk populations? Is there a minimum level of contamination required to cause disease? What are the benefits, risks, gaps in implementation, and barriers to uptake of *Legionella* control programs?
- **Policy and Training Issues:** What policies, regulations, codes, or guidelines affect the incidence, control, quantification, and prevention of Legionnaires' disease? How might they be built upon to better protect the public? How can *Legionella* control be best balanced with other water priorities?

ORGANIZATION OF THIS REPORT

Chapter 2 discusses Legionnaires' disease, the life history and complex ecology of *Legionella* in both natural and built water environments, and common exposure pathways. Chapter 3 focuses on the surveillance of Legionnaires' disease in the United States, methods to detect *Legionella*, and the results of environmental *Legionella* monitoring in different built water systems. Chapter 4 considers the many strategies used to control *Legionella*, including the use of heat, biocides, flow control, plumbing materials, aerosol formation prevention, and distal devices, along with their application in several typical built environments. Finally, Chapter 5 takes on the array of laws, regulations, codes and standards, and guidance documents that relate to *Legionella* management, both in the United States and abroad. This final chapter makes suggestions for how these various policy tools can be strengthened to better protect the public from legionellosis. Each chapter ends with conclusions and recommendations that synthesize more technical and specific statements found within the body of each chapter. The most important conclusions and recommendations are repeated in this summary.

DIAGNOSIS, ECOLOGY, AND EXPOSURE PATHWAYS

Since its discovery in the 1970s, more than 61 species of *Legionella* have been described, half of which have been isolated from patients. In North America and Europe, *L. pneumophila* is the most dominant *Legionella* species isolated from patients. Other species can lead to disease, including *L. micdadei, L. bozemanii, L. dumoffi* and *L. longbeachae.* In Oceania and parts of Asia, disease due to *L. longbeachae* approaches or exceeds that for *L. pneumophila.* The various species of *Legionella* differ in their virulence, infectivity, and preferred growth conditions (e.g., protozoan hosts and environmental factors). Yet what is known about legionellae ecology is almost exclusively based on studies with *L. pneumophila.* Similarly, a troublesome aspect of Legionnaires' disease diagnosis is the overreliance on the urinary antigen test, which only detects *L. pneumophila* serogroup 1.

L. pneumophila can adapt to environmental change by differentiating into replicative, transmissive, filamentous, mature infectious, and viable-but-not-culturable-like (VBNC-like) cells—forms that differ in their infectivity and their response to water treatment technologies. Even more complicated is that as pathogenic legionellae grow to high concentrations in free-living protozoa, infectious bacteria may be released within aerosols in various forms: as free cells, cells within biofilm fragments, or cells associated with free-living protozoan trophozoites, cysts, or expelled vesicles. A deeper understanding of *Legionella* ecology and of the genetic traits that equip *Legionella* strains to colonize engineered water systems, to survive in aerosols, and to thrive in the human lung is required. The following conclusions and recommendations for research are found in Chapter 2.

There is a need to better understand the mechanistic pathways for the development of Pontiac fever, and what roles the pathogen, endotoxins, *Legionella*-harboring amoebae, or other exposures play in disease pathogenesis. Because Pontiac fever is associated with less mortality, focused studies examining this clinical entity have been limited to date. There is a need to develop improved diagnostic tools for Pontiac fever (including molecular methods) that would enhance overall *Legionella* epidemiology and outbreak investigation.

Protocols should be developed to generate, identify, enumerate, and report distinct *Legionella* cell types. The capacity of *L. pneumophila* to resist detergents, heat, chemical disinfectants, and antibiotics,

as well as predatory amoebae and white blood cells depends on its growth phase. The resilience and infection potential differ by orders of magnitude for replicative, stationary or transmissive phase, and the mature infectious form of *L. pneumophila*.

Whether *L. pneumophila* persistence within built water systems is promoted by the bacterium's differentiation into an apparent viable-but-non-culturable state that is both resilient and reversible remains an urgent question with implications for public health. To date, studies of VBNC-like *L. pneumophila* are largely descriptive. Protocols to generate and isolate pure populations of VBNC-like cells for physiological, biochemical, genetic, molecular, and infection studies are needed.

Ecological studies have almost exclusively focused on the impact of environmental conditions on growth, survival, and inactivation of *L. pneumophila*. To clarify whether the ecological principles observed for *L. pneumophila* also apply to other pathogenic *Legionella* species, research on the ecology of *L. longbeachae*, *L. micdadei*, *L. dumoffi,* and other pathogenic *Legionella* species is warranted. The ecological conditions responsible for *L. pneumophila* growth in environments such as cooling towers, wastewater treatment plants, soils, and hot springs are largely unexplored compared to building water systems (i.e., premise plumbing).

Whether legionellae persist within free-living protozoa versus growing to high numbers appears to be influenced by many poorly understood factors, including temperature, species of bacterial prey available, presence of host symbionts, and host cell form. **Direct observations and metagenomic studies of microbial diversity are required to identify the protozoa that control the growth of pathogenic *Legionella* in various environments.** Microcosm studies could investigate how nutrients and biocides affect the life stages of the host protozoa (e.g., by triggering encystation), identify the key host species, and elucidate the role of other free-living protozoa that might feed on the primary hosts of legionellae.

QUANTIFICATION OF *LEGIONELLA* AND LEGIONNAIRES' DISEASE

Chapter 3 addresses Legionnaires' disease rates from surveillance systems and the occurrence of *Legionella* bacteria in various water systems including the methods used to detect *Legionella* in clinical and environmental samples. Monitoring of both Legionnaires' disease and *Legionella* bacteria in the environment is fraught with difficulties, including which pneumonia patients are tested to diagnose the cause of their infection, where and when to sample in the environment, what detection methods to use, and how to interpret the data. More and improved environmental monitoring is needed to examine (1) the national occurrence of Legionnaires' disease and *Legionella* concentrations in different built environments, (2) the environmental conditions and amplification niches for the bacteria, and (3) the sources of exposure for both sporadic and outbreak-associated Legionnaires' disease.

Surveillance data show that Legionnaire's disease rates have been rising in the United States and in Europe over the past 20 years. Current incidence rates are understood to be a substantial underestimate of the actual disease burden for many reasons, including the lack of adequate diagnostic testing among pneumonia patients in most U.S. hospitals and the virtual absence of diagnosis for outpatients. Using data from previous studies and current surveillance systems, **the Committee estimates that the number of persons with Legionnaires' disease ranges from 52,000 to 70,000 in the United States each year.**

By reviewing dozens of *Legionella* studies from various building types from around the world, the Committee found the available *Legionella* occurrence data are highly variable and sparse, making

comparisons among studies difficult and detection of spatial and temporal trends almost impossible. Available data suggest that cooling towers, hot tubs, showers, and wastewater treatment plants can be hot spots for growth of *Legionella* and exposures. Several studies that recorded concentrations of culturable *Legionella* were compiled to determine if and when concentration could be indicative of outbreaks of Legionnaires' disease. **A *Legionella* concentration of 5 x 10^4 colony-forming units per liter (CFU/L) should be considered an "action level", that is, a concentration high enough to warrant serious concern and trigger remediation. A lower action level may be necessary to protect those at higher risk for legionellosis, such as hospital patients, particularly those in intensive care, cancer, and solid-organ transplant units.** Additional conclusions and recommendations about Legionnaires' disease surveillance, environmental monitoring data, and quantitative microbial risk assessment are found below.

There is an urgent need to develop better clinical tools that will capture more cases of Legionnaires' disease and identify pathogenic *Legionella* beyond *L. pneumophila* serogroup 1. Hospitals in both rural and urban areas should have access to on-site urinary antigen testing to facilitate more targeted antimicrobial therapy and to increase disease recognition. Efforts to develop standardized molecular methods for *Legionella* diagnoses (including non-*pneumophila* species and serogroups other than serogroup 1) should be prioritized by research laboratories and federal agencies. Finally, the U.S. Department of Health and Human Services should target research funding to multi-center prospective studies of clinical respiratory samples using these new assays to better understand prevalence and diversity of the *Legionella* species and serogroups causing disease.

An improved understanding of sporadic, community-acquired cases of Legionnaires' disease is critical to reducing the rising rates observed over the past 20 years. **Determining the most common sources of sporadic disease will require well-funded, population-based studies in multiple jurisdictions (e.g., cities, counties, states).** Such studies would require the recruitment of multiple medical centers with an adequate number of *Legionella* cases each year, willingness and capacity to collect clinical samples for *Legionella* culture, personnel with knowledge of how to sample the most likely sources of exposure for legionellosis patients, and laboratory capacity to reliably grow *Legionella* from clinical and environmental samples.

Regional Centers of Excellence for prevention and control of legionellosis could serve as a backbone to strengthen the capacity of state health departments to detect and investigate cases of Legionnaires' disease. Such centers could be modeled on the Integrated Food Safety Centers of Excellence and the Centers of Excellence for Vector Borne Diseases, with modifications to include the relevant disciplines needed for *Legionella* applied research and control. These centers could promulgate best practices for prevention and control measures and they could train and assist building managers as they create water management plans. They could also help coordinate the in-depth, multiple-jurisdiction studies of environmental exposures recommended above.

A systematic study to compare culture methods for *L. pneumophila* (and other pathogenic legionellae) with quantitative polymerase chain reaction (qPCR), viability-qPCR, and reverse transcriptase qPCR is needed to determine comparability. qPCR and its variants offer a more rapid method to quantify *Legionella* in the environment, and could be used consistently to inform decisions on decontamination and restoration of affected systems, to investigate the bacteria's ecology and exposure pathways, and as a quality control method. With side-by-side comparisons of methods in a broad range of settings, it may be that PCR-based or other simplified methods or test kits could be shown to be useful predictors of human health risk and adequacy of remediation.

There is a good framework to perform quantitative microbial risk assessment (QMRA) for various *L. pneumophila* exposures. QMRA can be used to determine *Legionella* concentrations in building water systems that correspond to certain Legionnaires' disease risk levels; such information can be used, for example, to inform design and permitting decisions about pipe length, setback distances for large industrial cooling towers, and building-level hydraulic design to maintain acceptable microbial quality. To further advance QMRA, additional knowledge is needed about the impact of virulence and strain differences, phenotypic alterations in potency and aerosol survival, and generation rate of aerosols from various devices. Data on exposures, especially for cooling towers, are lacking.

STRATEGIES FOR *LEGIONELLA* CONTROL AND THEIR APPLICATION IN BUILDING WATER SYSTEMS

Chapter 4 focuses on strategies for *Legionella* control in building water systems. The controls considered are temperature control, disinfection, hydraulic management, nutrient limitation, choice of plumbing materials, distal devices, and prevention of aerosols. The chapter then discusses how specific controls are applied to building water systems, considering large engineered systems such as potable water supply, wastewater treatment, and reclaimed water systems, large institutional buildings and households, cooling towers and humidifiers, and hot tubs. The chapter also discusses several emerging issues, such as potential conflicts among strategies for green building design, water and energy conservation, and more prospective *Legionella* control strategies.

For any given building water system, multiple strategies can be successfully employed and should be used. The effectiveness of many of the controls are interdependent; for example, optimal hydraulics is required for effective delivery of thermal control and chemical disinfectant while reactivity of the plumbing materials and the water source chemistry could lead to disinfectant decay. Different strategies available for controlling *Legionella* in water systems are feasible at different stages of a building's life cycle, with some being most important during initial construction (e.g., the choice of plumbing materials) while others are implemented during ongoing operation and maintenance (e.g., disinfection and flushing). The conclusions and recommendations below highlight key takeaways with respect to *Legionella* control strategies for various building and device types.

For all types of buildings, hot-water heater temperature should be maintained above 60°C (140°F), and the hot-water temperature to distal points should exceed 55°C (131°F). Maintaining temperature outside *Legionella's* preferred growth range is the paramount *Legionella* control strategy for all buildings that provide hot water and has been proven successful by numerous longitudinal field studies. Temperature control is most effective in large, complex hot-water systems that are hydraulically balanced, with dead-end pipes removed and faulty devices that compromise the distribution of hot water identified and replaced.

There is growing evidence that, compared to free chlorine, a monochloramine residual better controls *Legionella* risk from building water systems, although the reasons for the improved performance are not yet clear. It is possible that amoebae trophozoites are more sensitive to monochloramine, causing the amoebae to encyst and thus preventing the proliferation of *Legionella* within their host. Additional research is needed to examine the precise action of monochloramine on *Legionella* persistence and growth within pipe biofilms.

Research is needed to better understand the persistence of distribution system disinfectant residuals within building plumbing. Public water supplies that maintain a disinfectant residual and manage hydraulics to prevent stagnation are helping to reduce *Legionella* exposure from the distribution system. Nonetheless, it is unclear to what extent the disinfection residual can achieve *Legionella* control within premise plumbing, for both single-family homes and small buildings as well as larger buildings.

Guidance about *Legionella* is needed for homeowners, especially consumers from at-risk segments of the population. In particular, there is a need to identify plumbing configurations and devices that inadvertently increase risk of *Legionella* proliferation as well as accessible, practical control options such as flushing taps after periods of disuse. Residential water systems can benefit from most of the control strategies discussed in Chapter 4, yet they are almost never formally implemented because of a lack of understanding or awareness on the part of homeowners and occupants.

Low-flow fixtures should not be allowed in hospitals and long-term care facilities because of these buildings' high-risk occupant populations. Low-flow fixtures have been promoted to conserve water and, in some cases, energy. Because of their lower flow, however, these fixtures, primarily low-flow faucets but also showers, increase water age and restrict disinfectant levels, including the disinfection provided by elevated water temperatures. As such, low-flow fixtures present a greater risk for *Legionella* development in the plumbing systems that feed them.

New designs are needed to help advance control of *Legionella* in cooling towers and humidifiers. Humidifier designs that produce water droplets within the temperature range conducive to *Legionella* growth should be avoided for use in new buildings, and existing units of these types should be replaced during building renovations. Strategies relying on disinfectants should consider using alternate types of biocides at regular intervals, since bacteria can regrow in cooling towers when biocide use is infrequent and irregular. Finally, cooling tower manufacturers should collectively design new systems that can operate at condenser water temperatures whereby the temperature going to the cooling tower will be greater than 60°C.

Green buildings have exacerbated many of the problems with *Legionella* by lengthening water residence times (which leads to loss of disinfectant residual) and lowering hot-water temperatures in premise plumbing. Criteria for certifying green buildings, energy-conserving features, and water-conserving features should be modified to take into account risk factors for *Legionella* growth. Substantial water conservation can still be potentially achieved while protecting public health with more overt management of water age, for example, through routine flushing.

REGULATIONS AND GUIDELINES ON *LEGIONELLA* CONTROL IN WATER SYSTEMS

Unlike Australia, Canada, and many European countries, the management of *Legionella* in water systems in the United States occurs on an *ad hoc* basis, ranging from no requirements at all to regulations that require some buildings to have water management plans that include *Legionella* monitoring of water samples along with treatment. The federal Safe Drinking Water Act does not provide any substantial control of *Legionella* in water systems.

Regulations in the Unites States that affect *Legionella* management (by requiring water management plans or monitoring of water systems for *Legionella*) currently cover healthcare facilities in New

York State, cooling towers in New York City and New York State, healthcare facilities within the Veterans Health Administration, and hospitals and healthcare facilities receiving Medicare or Medicaid funds. All other buildings and private residences are formally protected from *Legionella* only through the application of building and plumbing codes. The following recommendations are made to develop a more comprehensive policy for *Legionella* management in the United States.

Expand the Centers for Medicare & Medicaid Services (CMS) Memorandum to Require Monitoring for *Legionella* in Environmental Water Samples. The CMS memo of 2017 requires that hospitals and long-term care facilities receiving CMS funding develop and implement water management plans. This memo has appropriately targeted buildings in which the mortality rates of Legionnaires' disease are high because of the vulnerable patient population. Routine quantitative *Legionella* monitoring programs would enable these institutions to assess the effectiveness of their water management programs. Such enhanced data collection from within hospital systems could help refine the data thresholds needed for prevention. This emphasis on *Legionella* monitoring is supported by international regulations, by the Veterans Health Administration (VHA) directive and the New York State regulations, and by guidance from the American Industrial Hygiene Association (AIHA).

Register and Monitor Cooling Towers. Regulations and guidelines requiring the registration of cooling towers provide a demonstrable public health benefit with minimal regulatory burden to building owners and managers. Cooling tower registries enable a rapid public health response to community clusters of legionellosis cases, including timely remediation of possible sources of infection, and they can also be used to assess the contribution of cooling towers to overall disease incidence. In addition, regulations requiring ongoing *Legionella* monitoring of cooling towers have been shown to reduce cooling tower colonization rates in jurisdictions where they have been implemented (e.g., Quebec and Garland, Texas).

Require Water Management Plans in All Public Buildings Including Hotels, Businesses, Schools, Apartments, and Government Buildings. The standard of care specified for water management plans should be considered best management practice for all public buildings. The recommendation here is to codify what are currently voluntary standards for managing public buildings. ASHRAE 188, AIHA (2015), and other guidance documents are available to help create a water management plan that can meet this requirement. Ideally, this requirement would be codified by either local jurisdictions with authority (such as building inspectors) or state authorities (such as departments of environmental protection or health). Once codified, the requirements could be supported by insurance companies; that is, without a water management plan, a building would not qualify for insurance.

Require a Temperature of 60°C (140°F) at Hot-Water Heaters and 55°C (131°F) to Distal Points. Optimal operating temperatures at critical points in the hot-water system are based on an international consensus that maintaining minimum temperatures across the different parts of a hot-water system is the first barrier to implement to restrict *Legionella* growth. Monitoring temperature at the distal points of hot-water systems would be necessary to verify that this requirement is being met. These temperature requirements could be codified by changing building and plumbing codes or by modifying the CMS memo. There is also the possibility of these requirements being incorporated into guidance documents as they undergo revision in the future.

The recommendations discussed above are not prioritized; accomplishing any one of them would lead to important legionellosis risk reduction, with a cumulative effect as more of them are accomplished. The recommendations differ substantially in their necessary implementation schedule, which entities would provide oversight, their cost, and what other capacities need to be in place to support them. In particular, the additional monitoring requirements will necessitate the development of guidelines to interpret monitoring data. QMRA could play an important role by, for example, being used to develop routine operational targets for different types of building water systems to determine that the risk of legionellosis is acceptably low. Finally, it will be important to expand training and education on legionellosis and on the prevention and control of *Legionella* amplification in water systems. Education and training are particularly needed for those designing water systems, those overseeing municipal water supplies, those developing and implementing plumbing codes, those responsible for maintenance of water operations and premise plumbing, and those in government who are responsible for the safety of buildings, cooling towers, and the potable water supply.

1
Introduction

The leading cause of reportable waterborne illness in the United States today is Legionnaires' disease, a pneumonia caused by the *Legionella* bacterium. *Legionella* was first documented as a cause of human disease in 1976, after an outbreak of pneumonia of unknown origin was described among members of the American Legion who had attended a conference at the Bellevue-Stratford Hotel in Philadelphia. Of the nearly 2,000 conference attendees, 182 people developed clinical disease and 29 died from their illness. This large outbreak generated national alarm, as public health experts, laboratory scientists, and clinicians raced to define the pathogen (Winn, 1988). A subsequent epidemiological investigation revealed a relationship between the attack rate and the time spent in the hotel lobby and consequently, the route of exposure was surmised to be airborne. The high attack rate coupled with disease severity may have reflected the prevalence of pre-existing conditions among those exposed: of the 94 hospitalized cases, 58 had pre-existing conditions (Fraser et al., 1977). Once the etiologic agent was identified, antibody titers of hotel employees suggested they had been exposed to the bacterium intermittently over a long period.

In 1978 Dr. Joseph McDade and colleagues at the U.S. Centers for Disease Control and Prevention (CDC) discovered the etiologic agent of the Philadelphia outbreak (McDade et al., 1979). The bacterium responsible was named *Legionella pneumophila*, reflecting the patients initially diagnosed with disease and the respiratory complications seen with infection. The first to isolate *Legionella* species (spp.) is thought to be Hugh Tatlock in 1943 (Tatlock, 1944); subsequently, in 1959 F. Marilyn Bozeman isolated these bacteria from individual human cases. Retrospectively, the Philadelphia infectious agents were determined to be similar to the *L. pneumophila* seen previously by Bozeman in 1959 (Bozeman et al., 1968), as judged by immunological assay and similarity of guanine-cytosine DNA composition (McDade et al., 1979).

As news of the Philadelphia outbreak and the identification of *L. pneumophila* as a human pathogen spread, legionellosis became recognized throughout the world. *Legionella* spp. were retrospectively linked to previous enigmatic outbreaks of respiratory disease, including one dating back to 1957 in Minnesota. Thus, legionellosis was present in the United States for years prior to its detection in 1978 (Osterholm et al., 1983; Thacker et al., 1978). Another retrospective study linked *L. pneumophila* to a previously undiagnosed cluster of patients in a county health department facility in Pontiac, Michigan, struck by a "flu-like," less severe form of the illness associated with fever, headaches, and myalgias—a syndrome termed Pontiac fever (Glick et al., 1978). As culture techniques were improved and adopted by the microbiology community, the number of reported *Legionella* spp. grew to include *L. micdadei*, *L. bozemanii*, and *L. longbeachae*, among others. At the time of this publication, more than 61 *Legionella* spp. have been identified, of which more than half are associated with human disease (Cunha et al., 2016).

In the period following the Philadelphia outbreak, knowledge surrounding legionellosis expanded rapidly (see Figure 1-1). In the first few years after the outbreak, scientists identified the pathogen, procedures critical for laboratory isolation, common environmental sites of exposure (e.g., air conditioning units, cooling towers, potable water), and the underpinning to new methods for diagnosis (e.g., the urinary antigen test) (Berdal et al., 1979; Feeley et al., 1978; Politi et al., 1979; Shands et al., 1985). Importantly, epidemiologic and laboratory animal models demonstrated the benefits of macrolides and flouroquinolones as antibiotic therapy for legionellosis (Fraser et al., 1978). Epidemiologic studies also identified *Legionella* spp. as primarily waterborne pathogens. In the 1976 Philadelphia outbreak, the hotel's cooling tower was the source of water droplets contaminated with *Legionella* that spread through the air in and around parts of the hotel. Subsequently, many *Legionella* outbreaks have been linked to water exposures, including clusters caused by potable water sources in hospitals (Broome et al., 1979; Tobin et al., 1980, 1981). A timeline of *Legionella*-related events since 1943 is provided by Figure 1-1. Medical and epidemiological terms related to Legionnaires' disease and used throughout this report are defined in Box 1-1.

CLINICAL DISEASE AND EPIDEMIOLOGIC STUDIES

Legionnaires' disease is caused by bacteria of the *Legionella* genus—small aerobic Gram-negative rods that are facultative intracellular pathogens. Humans are primarily exposed to *Legionella* through inhalation into the respiratory system, after which the organism replicates in pulmonary macrophages and monocytes. Incubation periods are thought to range from two to twelve days, but may be longer, particularly in immunosuppressed patients. Patients with *Legionella* pneumonia have fever, cough, shortness of breath, and myalgias (i.e., soreness or aching of the muscles)—common symptoms in other respiratory infections. Unlike most people with community-acquired pneumonias, however, patients with Legionnaires' disease more frequently have gastrointestinal symptoms and altered mental status and neurologic abnormalities. Patients with Pontiac fever present with fever, myalgias, chills, and headache, but by definition do not have pneumonia; most patients recover without treatment. Because both diseases have symptoms that are similar to other infections, the legionellosis diagnosis may be delayed or missed, which can lead to severe consequences in those with *Legionella* pneumonia.

Legionellosis is most common among the elderly and those who are immunosuppressed. Incidence is also higher in men and in people who smoke cigarettes. While disease from non-*pneumophila Legionella* spp. is more common in immunosuppressed patients, the majority of reported cases are caused by *L. pneumophila* and most frequently serogroup 1 (although this varies by country). *Legionella* spp. have been found on every continent (Aranciba et al., 2014; Beauté, 2017; Carvalho et al., 2008; Chaudhry et al., 2017; Chedid et al., 2005; Guo et al., 2015; Wolter et al., 2016; Yu et al., 2002), but most currently available epidemiologic data focus on legionellosis in large metropolitan areas in developed regions. In the United States, incidence of Legionnaires' disease increased more than five-fold from 2000 to 2017 (see Figure 1-2).

Legionnaires' disease is acquired by exposure to contaminated aerosols of water generated by manufactured devices such as showerheads and faucets, cooling towers, fountains, hot tubs, and other building water systems. Despite numerous reports of common-source outbreaks in the community, through travel or through hospital exposures, and despite improvements in epidemiologic and laboratory tools, the vast majority of *Legionella* cases remain sporadic, community-acquired cases for which the primary exposure source is never identified.

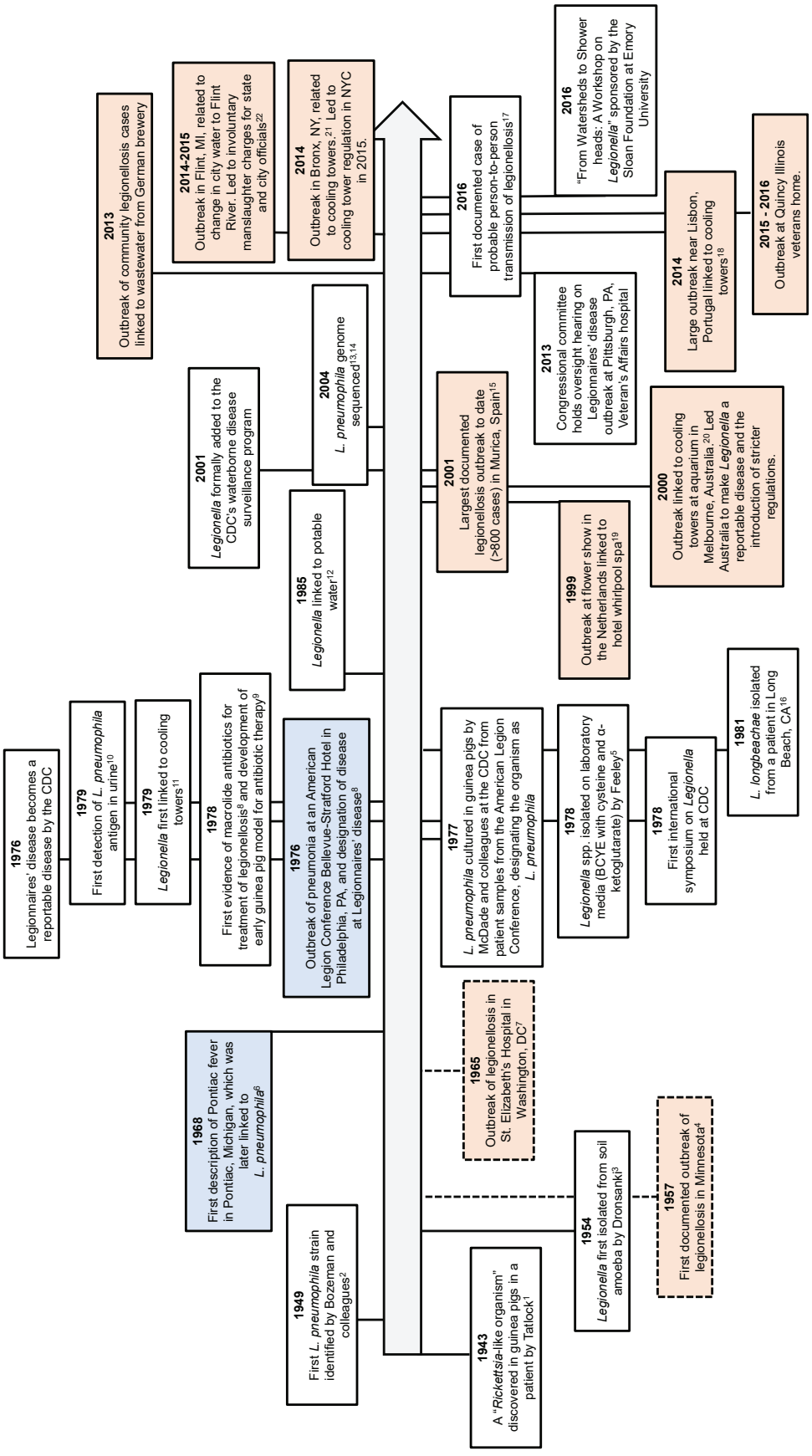

FIGURE 1-1 Timeline of important *Legionella* related events.
NOTES: Broken lines are outcomes determined through retrospective analysis. First *Legionella* spp. reported by Tatlock was *Tatlockia micdadei*, a synonym for *Legionella micdadei*. Blue indicates first reported cases of *Legionella* pneumonia and Pontiac fever respectively; orange indicates a large and historically significant outbreak. Citations indicated are listed at the end of the chapter. Timeline not to scale.

BOX 1-1
Legionnaires' Disease Definitions

Attack rate: The proportion of exposed people who become ill with (or who die from) a disease in a population initially free of the disease.

Case: An individual with a specified disease, illness, or condition who meets specific clinical, laboratory, and/or epidemiologic criteria.

Cluster: Cases of a disease, illness, or other health-related condition, grouped together in time and/or place.

Disability-adjusted life years (DALYs): A common health metric used to quantify the number of healthy years lost to disability, illness, and/or death due to a particular disease. Often used to compare the health burden of different diseases or in diseases among specific populations and to inform public health policy or decisions.

Disease burden: Measure of a health problem's impact that combines financial cost, mortality, morbidity, and/or other indicators.

Incidence: The occurrence of new cases of a given medical condition or illness in a population within a specified period of time.

Legionellosis: Disease caused by *Legionella* bacteria. Legionellosis includes both lung infections (Legionnaires' disease) and milder syndromes (Pontiac fever).

Legionnaires' disease (also known as *Legionella* pneumonia): Lung inflammation caused by *Legionella* infection, in which the lung's air sacs fill with inflammatory cells fighting the infection. Patients with pneumonia have evidence of lung involvement on radiologic images and have positive cultures or other tests (e.g., urinary antigen) for *Legionella*.

Mortality rate: a measure of the frequency of occurrence of death in a defined population (e.g., all individuals diagnosed with a specific disease) during a specified interval.

Nosocomial: A disease originating or acquired in the hospital, most commonly in reference to infections.

Outbreak: The occurrence of cases of disease or illness in excess of what would normally be expected in a defined community, geographical area, or season (World Health Organization definition).

Pontiac fever: A self-limiting "flu-like" illness caused by exposure to *Legionella* bacteria, defined by lack of pneumonia. Named after site (Pontiac, Michigan) of first description.

Sporadic: A disease or illness that occurs infrequently and irregularly. With regard to *Legionella*, those cases not associated with an outbreak.

Waterborne disease: A disease transmitted or propagated by contaminated water.

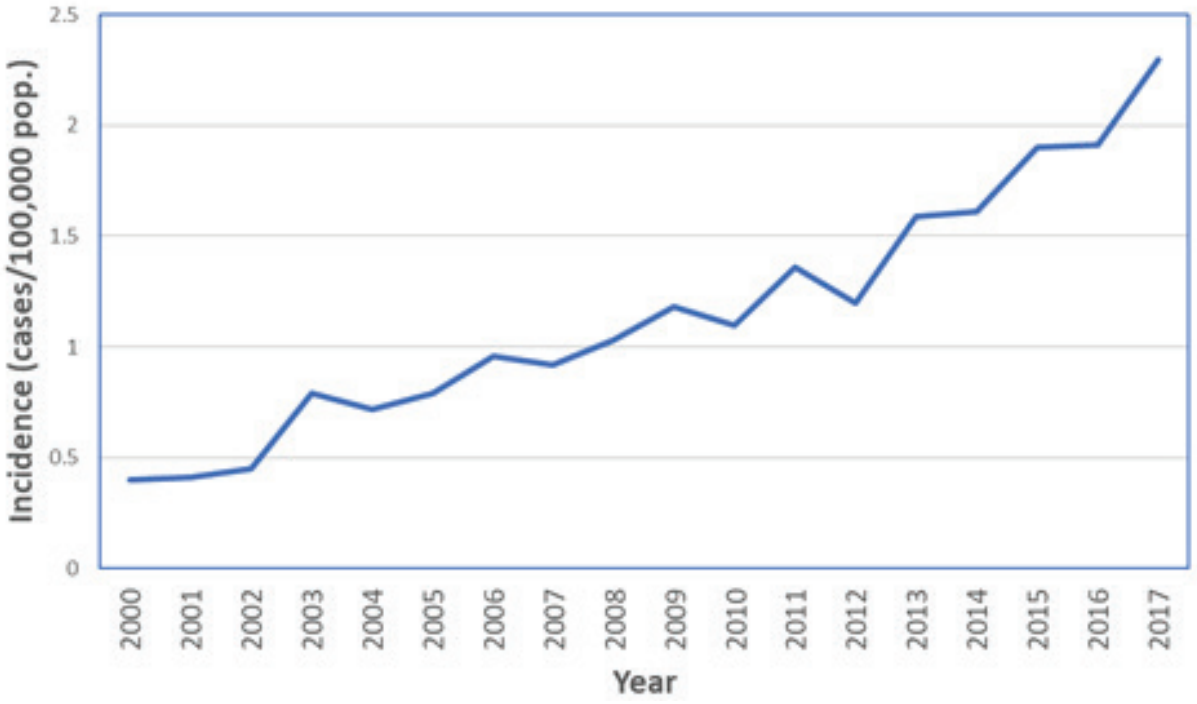

FIGURE 1-2 Increasing incidence of legionellosis in the United States from 2000 to 2017. SOURCE: Adapted from Shaw et al. (2018) with 2016 and 2017 data from the National Notifiable Disease Surveillance System (NNDSS).

The medical establishment has not optimized the diagnosis of Legionnaires' disease for many reasons. When patients present with pneumonia, most physicians choose empiric therapies that adequately treat the disease, so these cases are never counted. If a patient is tested using the urinary antigen test on site, results for infections caused by *L. pneumophila* serogroup 1 can be received in one day. However, when a patient is culture-tested for *Legionella*, it can be at least a week or longer before the results are known, making it difficult to diagnose cases in a timely manner. Worldwide, the actual burden of Legionnaires' disease is generally acknowledged to be underreported by as much as eight- to ten-fold (Dooling et al., 2015; Mercante and Winchell, 2015; Phin et al., 2014; St-Martin et al., 2013; von Baum et al., 2008).

Among common waterborne pathogens, *Legionella* is now the most common cause of reported drinking water-associated outbreaks (see Figure 1-3). Etiologic shifts from the 1970s to the modern era likely reflect successful efforts mandated by the Safe Drinking Water Act of 1974 to control fecal and enteric bacterial pathogens and parasites (primarily *Cryptosporidium* spp. and *Giardia lamblia*).

Waterborne infections account for $3 to 4 billion in excess costs in the United States per year (Adam et al., 2017). Nearly $1 billion per year goes to the top five primarily waterborne diseases (i.e., giardiasis, cryptosporidiosis, Legionnaires' disease, otitis externa, and non-tuberculous mycobacterial infections), including $430 million in hospitalization costs to Medicare and Medicaid (Collier et al., 2012). Proven *Legionella* cases are estimated to lead to a median cost of $26,000 to $38,000 per admission (Collier et al., 2012). European data paint a more ominous picture; Cassini and colleagues (2018) suggest that *Legionella* spp. are one of the top five pathogens leading to the most disability-adjusted life years (DALYs) and one of only four infections (including HIV, tuberculosis, and invasive pneumococcal disease) considered to have both a high population and high individual burden of disease (see Figure 1-4). Numerous sources worldwide have documented increasing incidence of *Legionella* cases, suggesting little progress in decreasing risk for *Legionella*. Incidence is generally thought to be underestimated, such that the true financial and human costs of legionellosis are also likely underestimated.

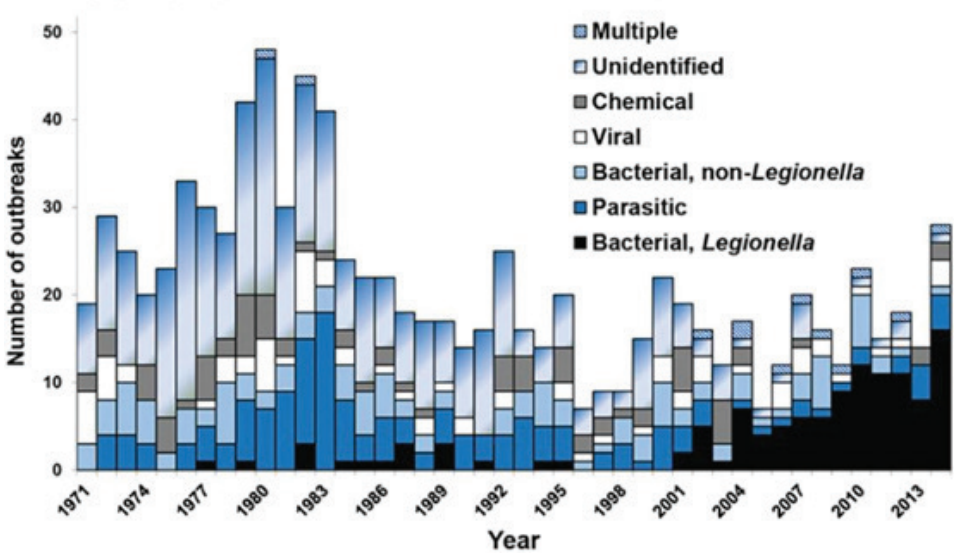

FIGURE 1-3 Etiology of reported drinking water associated outbreaks in the United States (n=298) by year, 1971 to 2014. SOURCE: Benedict et al. (2017).

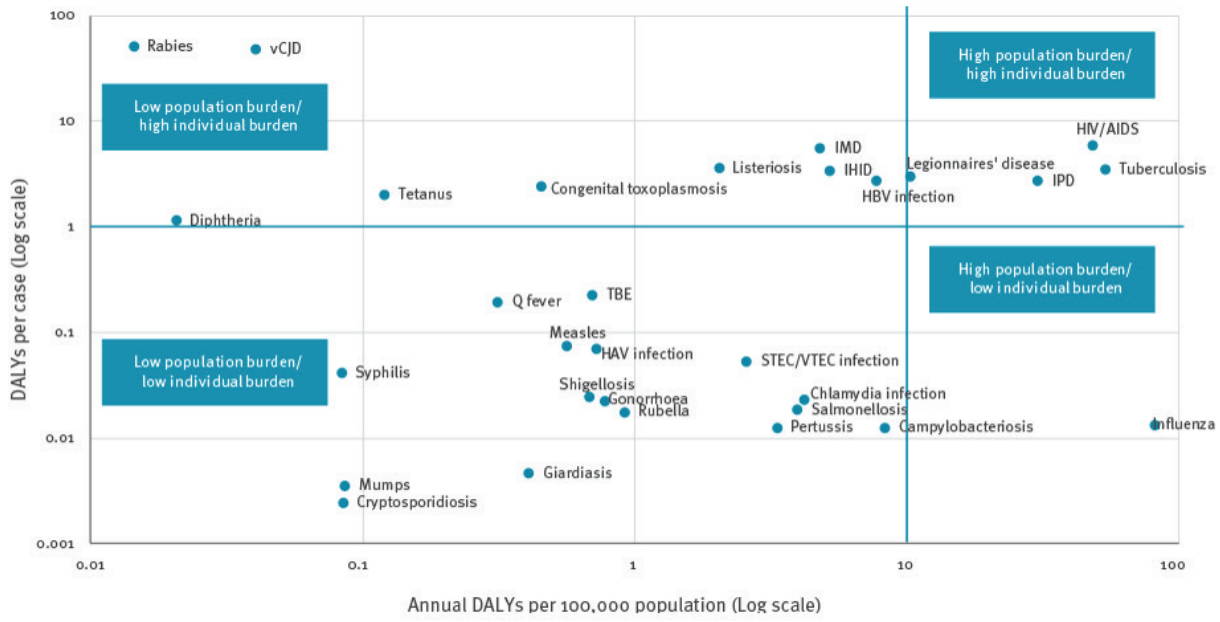

FIGURE 1-4 Scatterplot of the burden of selected infectious diseases in disability adjusted life years (DALYs) per case and DALYs per 100,000 population per year, European Union/European Economic Area countries, 2009 to 2013. SOURCE: Cassini et al. (2018).

NOTE: EU/EAA: European Union/European Economic Area; HAV: Hepatitis A virus; HBV: Hepatitis B virus; HIV/AIDS: Human immunodeficiency virus infection; IHID: Invasive *Haemophilus Influenzae* disease; IMD: Invasive meningococcal disease; IPD: Invasive pneumococcal disease; STEC/VTEC: Shiga toxin/verocytotoxin-producing *Escherichia coli*; TBE: Tick-borne encephalitis; vCJD: variant Creutzfeldt-Jakob disease. Diseases were arbitrarily subdivided according to burven in DALYs per 100,000 population and DALYs per case.

Table 1-1 lists waterborne bacterial pathogens associated with human sinopulmonary disease. Acquiring pneumonia from waterborne pathogens is uncommon, as most lead to gastrointestinal illness. Nonetheless, pneumonia can occur from waterborne pathogens, most notably *Legionella* spp., *Pseudomonas* spp., and non-tuberculous mycobacteria. Some of the listed bacteria grow opportunistically in building water systems, but only *Legionella* causes a reportable illness. Hundreds of organisms associated with waterborne disease are not discussed in detail in this report, including common waterborne pathogens such as *Pseudomonas* spp., non-tuberculous mycobacteria, *Campylobacter* spp., *Cryptosporidium,* and some *E. coli* infections. Nor does this report discuss other clinical illnesses linked to water, including otitis externa (an ear infection involving the external ear canal), diarrheal disease and their etiologic agents, and skin and soft-tissue infections, among others. Finally, Table 1-1 does not include a large number of important viruses, fungal spp., and parasites causing sinopulmonary infections that have been linked to water sources.

BUILT ENVIRONMENT IS A MAJOR ECOLOGICAL NICHE

Legionella bacteria naturally reside in many freshwater and soil environments, such as lakes, streams, and sediments, and many different species potentially cause disease. However, it is the unchecked growth of pathogenic legionellae in human-made water systems that typically leads to human exposures and causes disease. Humans are exposed to *Legionella* after inhaling or aspirating contaminated water aerosolized from a variety of sources.

L. pneumophila appears to grow poorly as individual, free-living cells in natural environments; instead, its growth is optimal within amoebae (Kuiper et al., 2004) and other free-living protozoa that are associated with biofilms (Buse et al., 2012; Hellinga et al., 2015). Indeed, the bacterial growth requirements are consistent with a natural, parasitic lifestyle. For example, replicating *Legionella* require external sources of certain amino acids and minerals (Reeves et al., 1981; States et al., 1985), low levels of oxygen (optimum below 1 mg/L) (Mauchline et al., 1992), and a temperature range between 25°C and 43°C (Garrity et al., 2005). During the *Legionella* life cycle, its physiological state switches between infectious and replicative forms as well as more hardy, dormant cell forms. Although *Legionella* can survive and persist in the absence of a host cell, in nature significant amplification appears to require protozoan hosts (Fields et al., 2002). Sometimes pathogenic legionellae replicate within free-living amoebae to levels that elevate risks to the people who are exposed (Ashbolt, 2015b; Declerck, 2010).

Accordingly, the ecology of *L. pneumophila* is directly linked to that of protozoa, whose primary habitat is biofilm (Declerck, 2010). A biofilm is a community of microorganisms within a self-produced hydrated gel matrix attached to moist soil, sediment, and other solid surfaces that accumulates organic and inorganic material (Characklis and Marshall, 1990). Biofilms typically form on all moist surfaces, including engineered surfaces such as pipes, tanks, appurtenances, filters, and gaskets—virtually everything that contacts water. Biofilm communities growing on pipes can include bacteria/archaea (including round, rod-shaped, filamentous, and appendaged forms), fungi, and higher organisms such as amoebae, ciliates, nematodes, larvae, and crustaceans (see Figure 1-5). The pipe material can exert a strong influence on the composition and activity of the biofilm's microbial community. Both surface materials and temperature influence the complex interactions among *Legionella*, host amoebae, and biofilm community members. (More detailed discussion on this ecology is found in Chapter 2.) In general, at moderate to warm temperatures, surfaces wetted with water that contains nutrients provide a favorable habitat for biofilm growth, grazing protozoa, and *Legionella* growing within the protozoa.

TABLE 1-1 Common Waterborne Bacterial Pathogens Associated with Human Sinopulmonary Disease

Pathogen	Associated Disease(s)	Source*	Comments
Acinetobacter spp.	bacteremia, pneumonia, skin and soft-tissue infections	Healthcare-acquired	• High resistance to chlorine (Karunmathil et al., 2014) • Associated with hospital outbreaks • Can grow in building water systems
Aeromonas spp.	bacteremia, pneumonia, skin and soft-tissue infections	Community-acquired	• Primarily *A. hydrophila* • Can grow in building water systems
Burkholderia cepacia complex	bacteremia, pneumonia	Healthcare-acquired	• Can grow in building water systems • More frequent in patients with weakened immune systems
Burkholderia pseudomallei	bacteremia, pneumonia, skin and soft-tissue infections	Healthcare-acquired	• Associated with hospital outbreaks • Resistance to multiple forms of disinfection • Can grow in building water systems
E. coli and other selected Enterobacteraciae spp.	bacteremia, pneumonia, diarrhea, gastroenteritis	Both	• Consider in community wells or if fecal contamination is present • Associated with groundwater and surface water contamination • Multidrug-resistant Enterobacteraciae associated with pneumonia in hospital water systems (e.g., *Klebsiella pneumonia*)
Legionella spp.	pneumonia	Both	• Common cause of outbreaks • Species vary by location and host; *L. pneumophilia* make up majority • Largest number of cases are community-acquired with no source identified • Can grow in building water systems • Non-water-based exposures can also lead to legionellosis • Pontiac fever patients do not have pneumonia
Methylobacterium spp.	bacteremia, pneumonia	Healthcare-acquired	• Primarily occurs in patients with compromised immune systems • Can grow in building water systems
Non-tuberculous mycobacteria	bacteremia, pneumonia	Both	• Primarily *Mycobacterium avium-intracellulare*, but others also reported (Falkingham, 2011) • Can grow in building water systems • High resistance to chlorine
Pseudomonas spp.	bacteremia, pneumonia, skin and soft-tissue infections	Both	• Primarily *P. aeruginosa* • Can grow in building water systems
Stenotrophomonas maltophilia	bacteremia, pneumonia, skin and soft-tissue infections	Healthcare-acquired	• Known intrinsic resistance to multiple antibiotics • More frequent in patients with weakened immune systems • Can grow in building water systems

*Probable source location designation (community or healthcare associated) is based on published data regarding documented water source links.

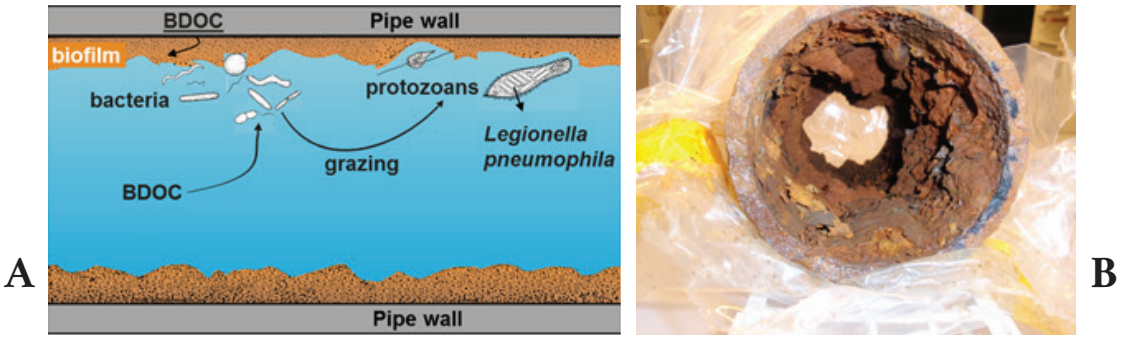

FIGURE 1-5 (A) Schematic of a biofilm growing on pipe walls, including the various microbial cells associated with the biofilm. (B) Corroded iron pipe showing the uneven surface that promotes the accumulation of biofilms. NOTE: BDOC =biodegradable dissolved organic carbon. BDOC can leach out of plastic pipe walls and can also be provided by microorganisms.
SOURCE: Courtesy of Paul van der Wielen.

The main mode of human exposure to *Legionella* is via inhalation of aerosols. Aerosols are small (typically <100 µm) drops of liquid formed by the action of turbulence on fluids, although only those less than 10 µm can reach deep into the human lung. Any materials suspended within the liquid, such as bacteria and protozoa, can be transported within these droplets. The aerosol particles have a large surface area-to-volume ratio and may selectively accumulate hydrophobic materials, including bacteria (Parker et al., 1983). Aerosolization is distinct from volatilization, which is a chemical phase change wherein either a dissolved solute, or the solvent itself, exits the liquid to form a true vapor state.

Sites with both biofilm growth and potential for aerosolization are possible sources of Legionnaires' disease risk. Many such areas exist in the built environment, including components of heating, ventilation, and air conditioning (HVAC) systems such as cooling towers and humidifiers; indoor plumbing (called premise plumbing) including outlets such as showerheads and faucets; and spas, hot tubs, and Jacuzzis (collectively called hot tubs in this report) (see Figure 1-6). Additional known sources of infection are fountains, misters, nebulizers, car washes, and industrial wastewater treatment plants.

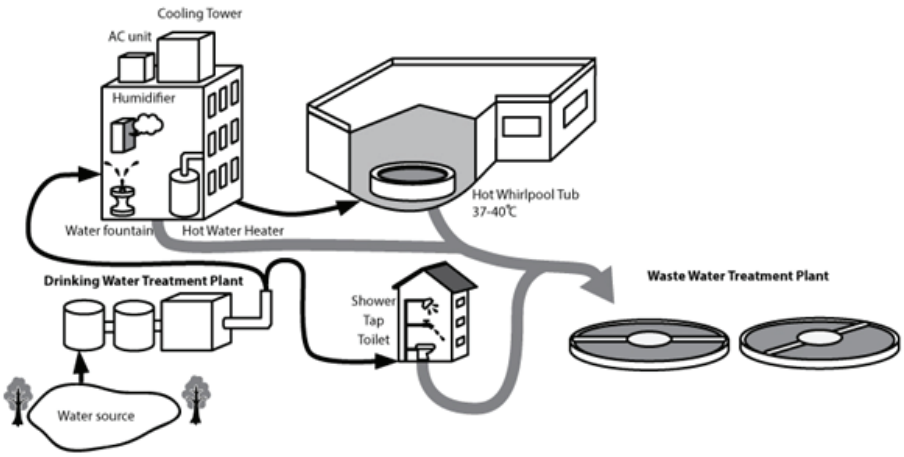

FIGURE 1-6 Built locations where *Legionella* growth can occur. NOTE: Thinner black arrows indicate water pathways to premise plumbing and thicker grey arrows indicate wastewater. SOURCE: Adapted from Exner (2018) by Kyoko Kurosawa.

Building- and industrial-scale (e.g., power plants, industries) wet cooling towers have been implicated in many outbreaks of Legionnaires' disease (see Figure 1-7). Cooling towers remove heat from recirculating water used in water-cooled chillers, heat pumps, air compressors and other equipment. Heat is rejected from recirculating water in the cooling tower primarily through evaporation. Under certain conditions, biofilms can develop within water-associated piping, heat exchangers and other component surfaces. Furthermore, the warm temperatures of the bulk water in cooling towers are also conducive to *Legionella* growth. These towers may generate bacteria-laden aerosols that drift away from the building or facility and then are inhaled by people working and living in the building as well as passersby. Exposure can also occur indoors if the downdraft from cooling towers is transported into building interiors, via air intakes or infiltration. In the United States, there are estimated to be two million cooling towers including both individual and industrial towers.[1]

Legionella can also contaminate drinking water, either in distribution systems or premise plumbing. In the United States, more than 322 million people are served by 152,000 public drinking water systems with more than 1.2 million miles of water supply mains. The total length of premise plumbing, which refers to all piping downstream of the service line connection and within buildings, is thought to be more than 6 million miles (NRC, 2006). Compared with the main distribution system, premise plumbing uses relatively long sections of small diameter tubing with about ten times more surface area per unit length (NRC, 2006). Thus, premise plumbing provides extensive interior surface area for biofilm growth. Moreover, because of stagnation, premise plumbing is frequently devoid of a disinfectant residual. If water in the pipes becomes enriched with pathogens such as *Legionella*, occupants may be exposed to aerosols created by showerheads or faucets (see Figure 1-8). Other home appliances such as hot-water heaters (see Figure 1-9) and humidifiers (see Figure 1-10) can also provide habitats for biofilm growth and enrichment. In 2018, there were estimated to be over 127 million households in the United States.[2]

Biofilm and *Legionella* growth can also be enhanced by water age, which depends on the building type and use, occupancy, and water use. Indeed, the water residing in premise plumbing has a much wider age distribution than the water entering a home from the distribution system (NRC, 2006). Although in the United States the average hotel occupancy is about 66 percent, it can fluctuate seasonally between less than 50 percent to more than 75 percent,[3] creating significant potential for water stagnation. There are over 5 million hotel rooms in the country[4]. Green buildings may provide additional areas for growth of pathogens because of lower hot-water temperatures, lower flows, and longer building water ages (Rhoads et al., 2016).

[1] See https://energytrendswatch.com/2017/11/21/cooling-towers-not-so-cool/.
[2] See https://www.statista.com/statistics/183635/number-of-households-in-the-us.
[3] See https://www.statista.com/statistics/206546/us-hotels-occupancy-rate-by-month.
[4] See https://www.statista.com/statistics/245864/us-hotel-rooms-by-chain-scale-segment.

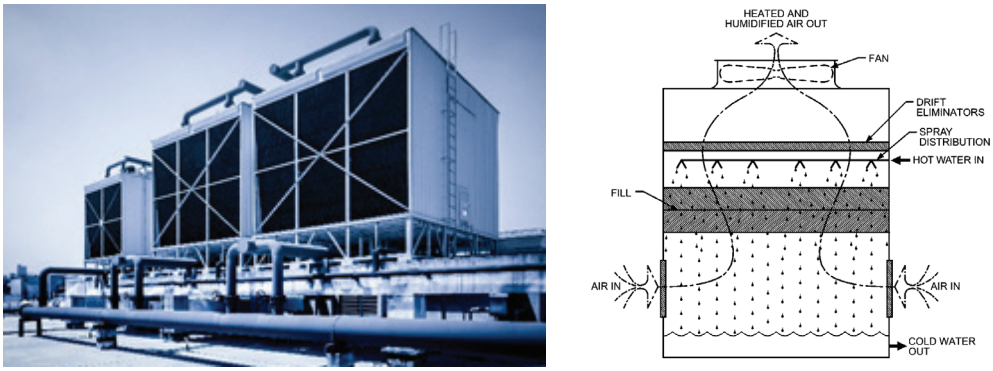

FIGURE 1-7 Photo and schematic of a cooling tower. SOURCES: Shutterstock and ASHRAE (2016).

FIGURE 1-8 A faucet and a fine-mist showerhead showing the potential for aerosolization. SOURCE: Shutterstock.

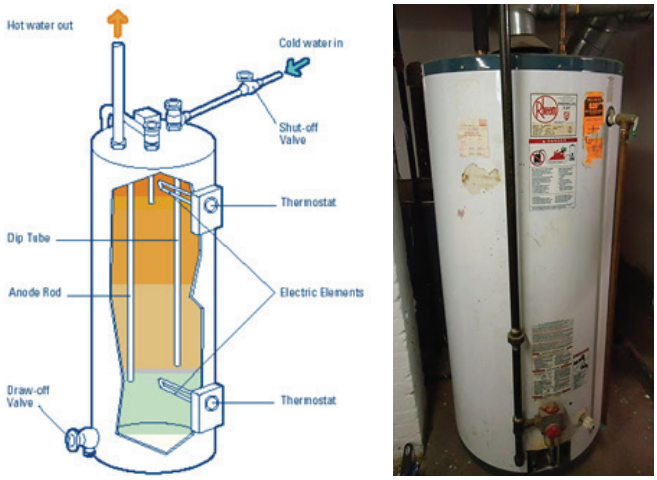

FIGURE 1-9 Schematic of an electric hot water heater and photo of a gas hot-water heater. SOURCES: https://buildingsfieldtest.nrel.gov/electric_resistance_water_heaters. © Government of Yukon (diagram) and WikiCommons (photo).

FIGURE 1-10 A hot tub, a humidifier, and a fountain. SOURCE: Shutterstock.

In addition to the premise plumbing and fixtures typical of residential and commercial buildings, healthcare and medical facilities provide additional opportunities for both biofilm colonization and aerosolization due to specialized medical devices, such as dental units and hydrotherapy units. Moreover, compared with the general public, their susceptible patient populations are at risk of Legionnaires' disease of greater intensity or severity. In the United States there are more than 6,200 hospitals, with more than 930,000 beds[5]. In 2015 there were about 1.7 million nursing home beds in the United States[6]. According to the American Dental Association, there are almost 200,000 dentists in the United States[7].

Another major type of built environment that can generate *Legionella* risk are recreational water features, both outdoor and indoor. These include swimming pools, hot tubs, hot-spring baths, fountains (see Figure 1-10), and water parks, as biofilms can form on surfaces and contaminated aerosols can be generated. Recreational sources have resulted in several Legionnaires' disease outbreaks (Leoni et al., 2018). In the United States, there are an estimated 9 million swimming pools and 5 million hot tubs.[8]

Other less common locales in the built environment can provide conditions suitable for biofilm growth, and hence colonization by pathogens such as *Legionella* and their protozoan hosts. Among these locales are interior water features such as green walls and waterfalls (den Boer et al., 2002; Haupt et al., 2012) and external building features that introduce additional wetted and irrigated surfaces including green walls or roofs, particularly when collected or harvested water is used for domestic purposes (Hamilton et al., 2017). Irrigating, lawn sprinkling, and spray washing with stagnant water from hoses or fixtures may be of concern as well, especially when using recycled water treated to non-potable standards (Hamilton et al., 2018; Johnson et al., 2018). In several countries, wastewater treatment plants receiving industrial wastewaters with temperatures higher than 25°C have also been identified as sources of Legionnaires' disease (e.g., Loenenbach et al., 2018; Olsen et al., 2010) and Pontiac fever (e.g., Castor et al., 2005; Gregersen et al., 1999).

ORIGIN OF THE STUDY

In the 40 years since the discovery of *L. pneumophila*, much has been learned about its ecology; in contrast, less progress has been made toward preventing Legionnaires' disease or understanding the ecology of different species of pathogenic *Legionella* and their protozoan hosts. Most knowledge regarding Legionnaires' disease comes from investigations of disease outbreaks, in which two or more people are infected at the same time by the same source. Yet, only 4 percent of Legionnaires' disease cases are associated with known outbreaks and are thoroughly investigated (Hicks et al., 2011). Whether outbreaks accurately represent the exposure route of the numerous sporadic cases that go unreported remains unclear. Thus, improved monitoring of Legionnaires' disease in patient populations and of *Legionella* presence in water systems is critical to better understanding the disease burden and the likelihood for particular water systems to be sources of infection.

Though monitoring the disease incidence and the presence of *L. pneumophila* in water samples has evolved considerably over the past 20 years, controversies associated with each persist. For Legionnaires' disease, a urine antigen test or a (more difficult) sputum test are considered diagnostic, and yet such tests are not commonly performed for hospital patients with pneumonia. Furthermore, the urine antigen test only detects one of the 14 known serogroups of *L. pneumophila.* Because of these reasons, among others, the number of cases of Legionnaires' disease is grossly underestimated in the United States as well

[5] See https://www.aha.org/statistics/fast-facts-us-hospitals.
[6] See https://www.statista.com/statistics/323196/number-of-licensed-nursing-home-beds-in-the-us.
[7] See https://www.ada.org/en/science-research/health-policy-institute/data-center/supply-and-profile-of-dentists.
[8] See http://www.apsp.org/Portals/0/2016%20Website%20Changes/2015%20Industry%20Stats/2015%20Industry%20Stats.pdf.

as in other countries that conduct surveillance. Water systems have traditionally been sampled using culture-based methods, which can take many days to detect growth and can be biased toward certain bacterial types. Polymerase chain reaction (PCR) methods exist, but their ability to differentiate between viable and nonviable organisms is still evolving. If routine monitoring of water systems for *Legionella* is to become standard practice for determining the risk of Legionnaires' disease for a given building or water system, accurate and quantitative microbiologic environmental testing is needed. Finally, although there is general agreement about the levels of detected *Legionella* that require remedial actions within a water system, there is no consensus on whether there is a threshold level of detected *Legionella* below which there is no risk of infection.

The treatment of water systems to reduce colonization by *Legionella* is further complicated by the bacterium's complex ecology. *Legionella* have developed multiple strategies to survive in the environment, including entering into a viable-but-non-culturable-like state, multiplying within a variety of protozoa including amoebae, and persisting within microbial biofilms. Indeed, its evolution of mechanisms to avoid digestion by its natural predatory hosts is thought to account for the virulence of certain strains of *Legionella* within human lung macrophages. Compared with bacteria in suspension, biofilms are relatively resistant to biocides and disinfectants, a primary means of treating built water systems. Other treatment methods involve raising water temperature beyond the growth range for the bacterium and host protozoa, reducing organic carbon levels in source water, altering pipe materials to discourage biofilm formation, and maintaining flow regimes in premise plumbing.

At best, a patchwork of laws, codes, policies, and guidance documents dictate how *Legionella* is managed in U.S. water systems and buildings. Healthcare facilities that are part of the Veterans Health Administration (VHA) system or that receive funding from the Centers for Medicare & Medicaid Services (CMS) must manage for *Legionella* contamination, but with varying requirements and oversight. Regulations to register, monitor, and treat cooling towers were put in place in New York City in the wake of the Bronx legionellosis outbreaks in the summer of 2015. New York State regulations now require all general hospitals and residential healthcare facilities to perform an environmental assessment, prepare and implement a sampling and management plan to test their potable water systems for *Legionella*, and institute control measures in the event of an exceedance. Similar and more widespread regulations for both cooling towers and buildings have existed in Australia, Canada, and some European countries for the past few years, and some are thought to have reduced the risk of Legionnaires' disease.

Beyond those buildings affected by the VHA and CMS requirements, management of legionellosis in the United States is mainly dictated via voluntary guidance documents from the American Industrial Hygiene Association, the American Society of Heating, Refrigerating, and Air-Conditioning Engineers, and the National Sanitation Foundation International, among others. Hence, *Legionella* management can be enforced only for a small subset of vulnerable buildings across the country. There is no federal law specifically targeting *Legionella.* The Surface Water Treatment Rule of the Safe Drinking Water Act only indirectly addresses *Legionella* via the requirement for maintaining a disinfectant residual in public water supply distribution systems that use surface water sources, and it does not extend to groundwater supplies or to building premise plumbing.

STUDY PURPOSE AND APPROACH

Following a May 2016 workshop on *Legionella* attended by representatives from around the world with expertise in public health, microbiology, and environmental engineering (Emory University, 2016), participants representing the National Academies of Sciences, Engineering, and Medicine sought to

commence a consensus study on *Legionella* that could address the shortcomings previously mentioned. CDC, VHA, the U.S. Environmental Protection Agency (EPA), and the Sloan Foundation provided the funding for a project to address the Committee on Management of *Legionella* in Water Systems' statement of task (see Box 1-2).

BOX 1-2
Statement of Task

There are many questions and gaps in information concerning *Legionella pneumophila* and Legionnaires' disease that the proposed National Academies of Sciences, Medicine, and Engineering project would confront. An ad hoc committee of the National Academies will review the state of science with respect to *Legionella* contamination of water systems and issue a report that addresses the following:

Ecology and Diagnosis: Describe the microbial ecology of water supplies (from the source to the tap and within built systems) as it relates to *Legionella*. What strains of *L. pneumophila* are of most concern and how can their diagnosis be improved (e.g., in terms of increased specificity, simplicity, and speed)?

Transmission via Water Systems: What are the primary sources and routes of human exposure to *Legionella*? What features/characteristics of water systems make them more or less likely to support growth of *Legionella*?

Quantification: Considering surveillance data, case studies of outbreaks, hospital data, other routine testing of water systems, what is known about the concentration of *Legionella* in water systems and the prevalence of Legionnaires' disease over the last 20 years? How uncertain are these estimates and what can be done to reduce this uncertainty? How can quantitative risk assessment be improved?

Prevention and Control: What are the most effective strategies for preventing and controlling *Legionella* amplification in water systems? What are the best methods to prevent exposure to *Legionella*, especially in at-risk populations? Is there a minimum level of contamination required to cause disease? What are the benefits, risks, gaps in implementation, and barriers to uptake of *Legionella* control programs?

Policy and Training Issues: What policies, regulations, codes, or guidelines affect the incidence, control, quantification, and prevention of Legionnaires' disease? How might they be built upon to better protect the public? How can *Legionella* control be best balanced with other water priorities?

Research: For the sections above, what additional information gaps exist and what knowledge must be gathered to fill these gaps?

ORGANIZATION OF REPORT

Chapter 2 discusses the diagnosis of Legionnaires' disease, the life history and complex ecology of *Legionella* in both natural and built water environments, and common exposure pathways. These active areas of research will require continued investment in order to improve the management of *Legionella* in water systems.

Chapter 3 focuses on the surveillance of Legionnaires' disease in the United States and Europe, as well as the environmental monitoring of *Legionella* that is becoming more common in built water systems. The need for a quantitative threshold of *Legionella* concentration above which action must be taken, and the role of quantitative microbial risk assessment, are extensively discussed in this chapter.

Chapter 4 considers the many strategies used to control *Legionella*, including the use of heat, biocides, flow control, aerosol formation prevention, and distal devices, along with their application in several typical built environments. The chapter also describes what is known about the efficacy of different control methods and their potential unintended consequences.

Finally, Chapter 5 reviews the array of laws, regulations, codes, standards, and guidance documents that relate to *Legionella* management, both in the United States and abroad. It includes suggestions for how these various policy tools can be strengthened to better protect the public from legionellosis.

Each chapter ends with conclusions and recommendations that synthesize more technical and specific statements found within the body of each chapter. The most important conclusions and recommendations are compiled in the report summary.

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2
Diagnosis, Ecology, and Exposure Pathways

Humans coexist with an abundance of microbes, organisms so small they are invisible to the naked eye. The vast majority are benign and many are beneficial, yet everyone can name microbes that cause disease. Although it is convenient to classify microorganisms as either friend or foe, such a distinction masks more complex interactions that dictate whether the human–microbe encounter promotes disease or health. In general, the impact of exposure to a particular microbe depends on the balance of three factors: the quantity of microorganisms, their capacity to cause harm, and the strength of an individual human's defenses (see Figure 2-1).

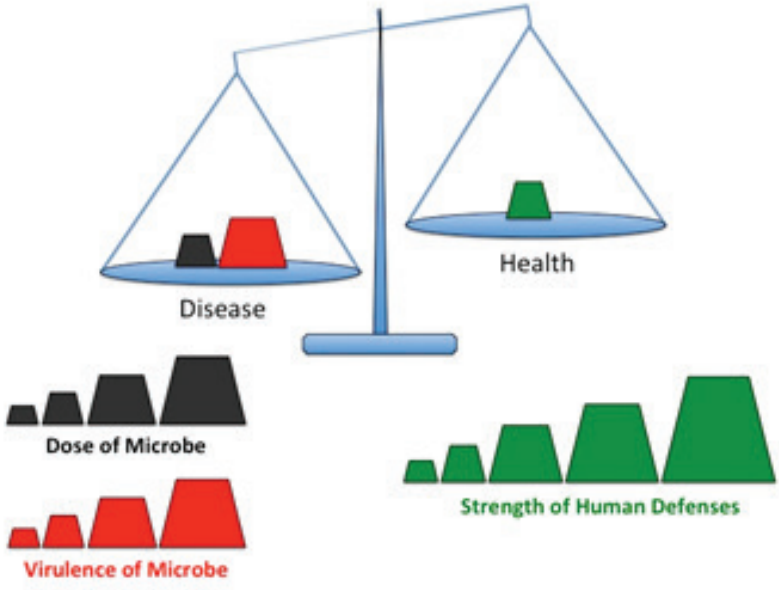

FIGURE 2-1 Consequence of exposure to microbial pathogens. The outcome of a host-microbe interaction depends on multiple factors: the dose, or quantity, of microbes; their virulence, or capacity to cause harm; and the strength of the human defenses. In the example shown, a robust immune response would tip the balance in health's favor. SOURCE: Adapted from Swanson et al. (2016). American Society for Microbiology, Copyright 2016; adapted with permission. No further reproduction or distribution is permitted without the prior written permission of the American Society for Microbiology.

To tip the balance toward infection, all microbes, including *Legionella*, must surmount multiple challenges. First, microbes need to encounter a susceptible host. For *Legionella*, aerosols of contaminated water or soil can disperse these bacteria, exposing people in the vicinity. Second, the microbe has to enter the host. When inhaled or aspirated, aerosols (less than 10 μm in diameter) can carry *Legionella* into aveoli in the lungs. Third, to initiate an infection, microbes must breach inborn defensive barriers. In the case of Legionnaires' disease, a respiratory tract damaged by cigarette smoke, for example, offers an increased opportunity to establish infection. Finally, to cause disease, the infecting microbe needs to inflict damage. The trauma may be direct, such as when a bacterial toxin punctures host cells, or an indirect effect of a hyperactive inflammatory response, for example. Legionnaires' disease injures lung tissue and protective lung macrophages, a consequence of not only factors released by virulent bacteria, but also a robust inflammatory response that wreaks collateral damage. Bearing in mind these four prerequisites to infection, one can understand why combinations of exposure, *Legionella* loaded with an arsenal of virulence factors, and/or impaired human immune defense barriers create opportunities for some *Legionella* strains to establish severe lung infections. How the interplay among host defenses, *Legionella pneumophila* biology, and the ecology of engineered water systems alter the balance between health and disease is the focus of this chapter.

HUMAN HOST

The majority of Legionnaires' disease cases (from 80 to 90 percent in Europe and the United States) are linked to *L. pneumophila* (Beauté et al., 2013; Cross et al., 2016; Dooling et al., 2015; von Baum et al., 2008; Yu et al., 2002). However, since its discovery in the 1970s, more than 61 species and 3 subspecies of *Legionella* have been described, half of which have been isolated from patients (e.g., Hazel et al., 1987; Jaeger et al., 1988; Khodr et al., 2016; Vaccaro et al., 2016). In people with weakened immune systems, species other than *L. pneumophila* that are frequently isolated include *L. micdadei, L. bozemanii, L. dumoffi* and *L. longbeachae* (Cunha et al., 2016; Rucinski et al., 2018). In Oceania and parts of Asia, disease due to *L. longbeachae* approaches or exceeds that for *L. pneumophila* (Whiley and Bentham, 2011).

Human and financial burdens due to *Legionella* are substantial. *Legionella* species (spp.), particularly *L. pneumophila*, can cost from $26,000 to $38,000 per hospital admission (Collier et al., 2012). More importantly, *Legionella* infections can be morbid, leading to prolonged hospitalization and often intensive care admission. Mortality rates of legionellosis range between 2.9 and 33 percent (Burillo et al., 2017; Cunha, et al., 2016; Gargano et al., 2017; Greenberg et al., 2006; Mykietiuk et al., 2005). Death is more likely for people who are immunocompromised (del Castillo et al., 2016; Han et al., 2015; Lanternier et al., 2017; Pedro-Botet et al., 1998; Sivagnanam et al., 2017), admitted to the intensive care unit (Chidiac et al., 2012; Cunha et al., 2016; von Baum et al., 2008), receive delayed antibiotics (Heath et al., 1996), or who develop hospital-acquired legionellosis (Jesperson et al., 2010; Soda et al., 2015; Stout et al., 2003).

As introduced in Chapter 1 and further explained in Chapter 3, *Legionella* pneumonia is a significantly underreported disease (Beauté et al., 2013; Jong et al., 2010; Neil and Berkelman, 2008). Incidence varies even when legionellosis is reported, likely because of differences in local ecology, water sources, temperature and weather patterns (Fisman et al., 2005; Ricketts et al., 2018), testing availability (Pierre et al., 2017), and surveillance of and methods for reporting incident cases. In the Etiology of Pneumonia in the Community (EPIC) study evaluating community-acquired pneumonia, *Legionella* pneumonia was the eighth most common pathogen identified for patients requiring hospital admission in five hospitals in Nashville, Tennessee, and Chicago, Illinois (Jain et al., 2015). Even this high ranking was conservative, as the EPIC study data only included assessment of *L. pneumophila* serogroup 1 and excluded high-risk

immunosuppressed patients or those with prior hospitalization. The Competence Network for Community-Acquired Pneumonia (CAPNETZ) study in Germany, which used culture, urinary antigen, and polymerase chain reaction (PCR) testing of samples, found that 97 out of 941 (9.6 percent) of all community-acquired pneumonia cases were due to legionellosis, the majority of which were attributed to *L. pneumophila* (von Baum et al., 2008). Non-*pneumophila* presentations of legionellosis, limited testing, use of empiric therapy, and lack of consensus for the epidemiological definition of presentations such as Pontiac fever (Tossa et al., 2006) further contribute to underreporting (Whiley et al., 2014). Furthermore, rates of disease caused by non-*pneumophila Legionella* spp. and non-serogroup 1 *L. pneumophila*, for which there are more limited diagnostic modalities, are also thought to be underestimated (Benin et al., 2002; Lode et al., 1987; Muder and Victor, 2002). Serological testing of blood donors indicates that exposure to *Legionella* may be higher than generally appreciated, with seroprevalence ranging between 4 and 22 percent and up to approximately 40 percent in some cities or among those with high-risk occupations (Borella et al., 2008; Coniglio et al., 2009; Nadaraja et al., 1987; Rudbeck et al., 2008; Valciņa et al., 2015).

Increasing Incidence

Studies worldwide have shown increasing incidence of *Legionella* cases (Beauté, 2017; Burillo et al., 2017; Neil and Berkelman, 2008). In the United States, reported cases increased five-fold from 2000 to 2017 (see Figure 1-2). The causes of this increase are not well characterized, but are thought to be multifactorial. Methods to detect *Legionella* in clinical samples are now both easier to perform (e.g., urinary assays) and more readily available outside of large academic laboratories, making their use increasingly common (Pierre et al., 2017). Community-acquired pneumonia national guidelines (Bradley et al., 2011; Mandell et al., 2007) and prediction tools (Cunha, 1998; Fiumefreddo et al., 2009; Miyashita et al., 2017) have both helped increase awareness of *Legionella*. However, these guidelines, aimed at community practioners, were created to streamline diagnostic work-up and antibiotic management. Indeed, most current guidelines recommend that even low-risk patients receive empiric antibiotics that include either a macrolide (in combination with a beta-lactam or cephalosporin or as the primary agent, depending on host risk factors) or a respiratory flouroquinolone (moxifloxacin or levofloxain) (Yu et al., 2004). These agents not only target many common causes of pneumonia, such as *Streptococcus pneumonia* and *Moraxella catarhallis*, but also cover atypical pneumonia pathogens including *Mycoplasma pneumoniae* and *Legionella* spp. Although such guidelines promote prompt administration of appropriate empiric therapy for patients with pneumonia, providers of such early antibiotic therapy may actually be less apt to pursue diagnostic testing for legionellosis. This tendency is particularly true for mild cases, as some guidelines only recommend testing for patients who have severe disease or are admitted to the intensive care unit (Mandell et al., 2007).

Changing demographics, such as the aging population, may contribute to the rise in disease incidence as well. From 1970 to 2018, the median age in the United States has increased by nearly ten years, such that Americans aged 65 and older now make up a larger portion of the U.S. population (CDC, 2013). And the number of elderly Americans is predicted to increase. Immune senescence of both innate and adaptive immunity plays an important role in increased risk among the elderly population (Boe et al., 2017). Enhanced survival of high-risk patients (e.g., those with an underlying condition such as cancer, cardiac disease, or lung disease) may also contribute to the increased incidence trend. Likewise, the increasing number of patients with compromised immunity due to immunosuppressive therapies and prolonged survival among higher-risk immunosuppressed patients may contribute to increasing legionellosis incidence. Although likely an underestimate, current data suggest that at least 2.7 percent

of Americans consider themselves immunosuppressed (Harpaz et al., 2016), indicating nearly 9 million at-risk individuals in the United States by this criterion alone. With the increasing number of agents that modify immune responses, improvements in survival, and the number of conditions that are currently treated with immunosuppressive therapy, this susceptible population is expected to increase. Specific immuncompromised populations are known to be growing. For example, the United Network of Organ Sharing reported a doubling of the number of patients receiving a solid organ transplant over the past 20 years[1]. Likewise, the National Cancer Institute estimates that the number of cancer survivors will increase by 30 percent over the next decade.[2]

Increasing population density in cities served by aging, centralized water systems (and including more cooling towers) may elevate the risk of legionellosis. Indeed, the American Society of Civil Engineers gave the U.S. water infrastructure a "D" rating (ASCE, 2017), noting that many pipes laid in the mid-20th century are now beyond their expected lifespan, increasing their risk for main breaks and intrusion, corrosion-enhanced biofilm development, and colonization with *Legionella*. Exposure in daily life may be more frequent due to increasing contact with water products and water devices, particularly those ideally suited for *Legionella* growth (e.g., cooling towers; hosing, faucets, and showerheads; hot tubs, Jacuzzis, and spas [collectively called hot tubs in this report]; humidifiers; fountains).

Additionally, climatic changes, including increased rainfall and global temperatures, have been linked to increasing disease incidence, either directly or through increased use of water sources linked to legionellosis. Most climate work has focused on the effects of temperature and rainfall events. Clear seasonality to *L. pneumophila* exposure has been established by multiple studies (European Centre for Disease Prevention and Control, 2013; Marston et al., 1994). For example, a PCR screen of more than 44,000 respiratory specimens over a recent eight-year period in Rochester, MN, documented annual peaks during the warm, humid months (Rucinski et al., 2018). Likewise, risk of disease increased during warm, wet periods in the mid-Atlantic region of the United States, as well as The Netherlands, Spain, and Taiwan (Chen et al., 2014; Fisman et al., 2005; Garcia-Vidal et al., 2013, reviewed by Walker 2018; Hicks et al., 2007; Karagiannis et al., 2009; Simmering et al., 2017). Cassell et al. (2018) suggests that precipitation is associated with the risk of sporadic Legionnaires' disease, noting a 48 percent increased risk of legionellosis two weeks after a 5-mm average increase in rainfall. With increasing global temperatures, more precipitation and flooding in some regions, and rising sea levels, there is concern that *Legionella* and other waterborne infections will continue to increase.

Worldwide, the actual burden of Legionnaires' disease is generally acknowledged to be underreported as a consequence of the generally low rate of diagnostic testing coupled with the reliance on a diagnostic test that is highly specific for a single serogroup of *L. pneumophila* (Dooling, 2015; Mercante and Winchell, 2015; Phin et al., 2014; St-Martin et al., 2013; von Baum et al., 2008). During a three-year period in Germany, about 10 percent of Legionnaires' disease patients were infected with species other than *L. pneumophila* (von Baum et al., 2008). Over a ten-year period in Denmark, 40 percent of the Legionnaires' disease cases that were confirmed by laboratory culture were caused by *L. pneumophila* that were not serogroup 1 (St-Martin et al., 2013). Similarly, in the United States, for the Legionnaires' disease cases reported from 2011 to 2013 to the Centers for Disease Control and Prevention's (CDC) Active Bacterial Core Surveillance network, 9 percent of the 140 culture-confirmed cases were due to non-serogroup 1 *L. pneumophila* (Dooling et al., 2015). Also troubling is the higher mortality among patients infected with these non-serogroup 1 strains compared with serogroup 1 *L. pneumophila* (Marston et al., 1994; Mercante and Winchell, 2015; St-Martin et al., 2013). These differences could be because of differences in those at risk for these pathogens or because of the lack of commonly available diagnostic tools, which delays identification. Finally, sole reliance on the urine antigen test hampers efforts to recognize

[1] See https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#.
[2] See https://www.cancer.gov/ about-cancer/understanding/statistics.

and interrupt outbreaks, as epidemiological investigations require discriminatory genetic tests of clinical and environmental *L. pneumophila* isolates (Mercante and Winchell, 2015).

Risk Factors for *Legionella* Disease

Numerous factors are linked to increased risk of legionellosis, varying from host factors to exposure factors. Indeed, in large outbreaks not everyone who is exposed to *Legionella* develops disease (Bartram, 2007; Phin et al., 2014). Male adults are at higher risk (Cunha et al., 2016; MacIntyre et al., 2018; WHO, 2018). Indeed, in the United States the incidence of reported cases of legionellosis in men (2.31/100,000) was approximately 50 percent higher than in females (1.50/100k) in 2016. Age is also commonly identified as a risk factor for legionellosis, with most studies suggesting risk begins increasing at approximately age 40 to 50 years (Bartram, 2007; Farnham et al., 2014; Sopena et al., 2007). Data on children are more sparse, but suggest that children may be less likely to develop severe infections (Greenberg et al., 2006; Muldoon et al., 1981; Yu and Lee, 2010). In one review of case reports, the majority of children who became ill had other at-risk diseases (e.g., cancer) and more than half were under the age of one (Greenberg et al., 2006). More than 70 percent of pediatric legionellosis cases may be hospital-acquired, suggesting either less clinical disease or underdiagnosis in the community (Alexander et al., 2008). Neonates may be at highest risk for hospital-acquired legionellosis because of both increased exposures and their weaker immune status (Levy and Rubin, 1998). Although infrequently reported, water births have been linked to some cases of neonatal legionellosis (Frazin et al., 2001; Ganseth et al., 2017).

Populations with jobs that increase occupational exposure to water are also at risk of legionellosis (Principe et al., 2017). Among these populations are water-service providers, maintenance workers, wastewater and cleaning personnel, and workers in industries that use industrial water sprayers (e.g., paper and textile mills, plastic molding factories).

Another classical demographic risk factor for legionellosis is impaired immunity, either through anatomic changes to the airway or weakened barriers to respiratory pathogens. Indeed, age-related immune senescence is thought to be an important reason why rates are higher among older patients. Smoking is a clear dose-dependent risk factor and possibly contributes to the higher incidence of cases reported for males than females. Smoking also changes the airway epithelium, perturbs pulmonary cilia function, decreases airway clearance, and alters the aerodigestive microbiome, factors each thought to increase the risk for bacterial pneumonias, including those caused by *Legionella* spp. (Arcavi et al., 2004; Gao et al., 2014; Morris et al., 2013). Not only are those who smoke at higher risk than those who have never smoked, but those who have smoked more than 20 cigarettes per day for more than 20 years have a risk 25 times greater than that of non-smokers as well as a higher incidence of disease during outbreaks (Che et al., 2008). Risk and severity of illness may be further increased by smoking-related complications such as chronic obstructive pulmonary disease or emphysema (El-Ebiary et al., 1997). Even exposure to second-hand smoke has been suggested to increase risk (Wang et al., 1995).

Another population vulnerable to *Legionella* pneumonia is patients at increased risk of aspiration, such as those with neuromuscular diseases, the elderly, and neonates (Blatt et al., 1993; Wei, 2014). (Indeed, studies suggest that episodes of silent aspiration are significantly more common among elderly patients when compared to age-matched controls [Kikuchi et al., 1994]). Researchers have hypothesized that the "microaspiration" that occurs during drinking or with particular clinical conditions may deliver *Legionella* to the lung and cause pneumonia (Lee and Ryu, 2018; Marrie et al., 1991). The oropharynx (Jaresova et al., 2006) and dental plaques (Tesauro et al., 2018) may also be colonized with *Legionella,* which would increase the likelihood of aspiration. Still, exposure pathways related to aspiration are

more difficult to investigate unless clearly linked to feeding tubes or other mechanical methods associated with tap water and aspiration (Dournon et al., 1982; Marrie et al., 1991; Muder et al., 1992; Venezia et al., 1994; Yu, 1993).

Patients with known impaired immune function, including those with organ dysfunction, are also at increased risk for legionellosis. Patients undergoing treatment for cancer and those who have received a solid organ transplant may be at highest risk because of the depth and length of immunosuppression required (del Castillo et al., 2016; Jacobson et al., 2008; Lanternier et al., 2017; Sivagnanam and Pergam, 2016). These patients are also at increased risk for non-*pneumophila* legionellosis and non-serogroup 1 *L. pneumophila* infections (Ampel et al., 1990; Dowling et al., 1984; Knirsch et al., 2000; Muder and Victor, 2002; Singh et al., 2004). Particular immunosuppressive agents have been linked to legionellosis, chiefly glucocorticoids such as prednisone (Htwe and Khardori, 2017). Patients receiving tumor necrosis factor inhibitors for rheumatologic and inflammatory bowel diseases are also at increased risk for legionellosis (Lanternier et al., 2013). Likewise, patients with renal dysfunction (including those on dialysis), liver disease, lung disease, and known cardiac dysfunction are more susceptible to legionellosis (Chidiac et al., 2012; Ongut et al., 2003; Viasus et al., 2013).namely high body temperature (OR 1.67, $p < 0.0001$ Immunosuppressed patients also have increased severity of disease including intensive care unit (ICU) admission, intubation, and death.

Genetic predisposition may account for enhanced susceptibility to disease from *Legionella* spp. in those with or without other risk factors (Berrington and Hawn, 2013). Legionellosis is linked to genetic polymorphisms in three human Toll-like receptors (i.e., TLR-4, TLR-5, and TLR-6), components of the innate immune system that recognizes pathogen-associated molecular pattern (PAMP) molecules (Hawn et al., 2003, 2005; Misch et al., 2013). Also associated with an increased risk for legionellosis is a common haplotype of the GMP-AMP synthase-stimulator of interferon genes (STING) pathway (HAQ TMEM173/STING), which is central for innate immune sensing of bacterial infections (Ruiz-Moreno et al., 2018).

Clinical Manifestations of Legionellosis

Legionella spp. cause clinically significant disease among susceptible human hosts. The most common manifestations are pneumonia (i.e., classical Legionnaires' disease) and Pontiac fever. Diagnosis can be challenging as many clinical signs and symptoms typical of legionellosis are often found in both infectious and non-infectious diseases (see Figure 2-2) (Cunha, 1998; Phin et al., 2014). More rarely, *Legionella* spp. have been associated with skin and soft-tissue infections, bacteremia, endocarditis, and septic arthritis (Banderet et al., 2017; Heriot et al., 2014; Kilborn et al., 1992; Pearce et al., 2011; Qin et al., 2002).

Worldwide, *Legionella* pneumonia is the most common manifestation of legionellosis. *Legionella* pneumonia is often classified as an "atypical pneumonia" along with those caused by bacterial pathogens such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. When compared with common bacterial pneumonia agents, atypical pneumonias may present common symptoms and radiologic findings and less common presentations (e.g., diffuse interstitial patterns on chest x-ray). Clinical and radiologic findings cannot distinguish between these pathogens and other causes of community-acquired pneumonia. However, they respond to antibiotic classes and agents that primarily target intracellular infections (Sharma et al., 2017). Following exposure, *L. pneumophila* has an incubation period of approximately two to ten days (Bartram, 2007). About 10 percent of cases have an incubation period longer than ten days, such that case information should be collected for a minimum of 14 days prior to onset of symptoms for community cases.[3] Likewise, incubation periods may be longer for hospitalized populations, which of-

[3] See https://legionnaires.ecdc.europa.eu/?pid=107.

FIGURE 2-2 Clinical symptoms of legionellosis: Pontiac fever and *Legionella* pneumonia (Legionnaires' disease). SOURCE: Courtesy of Kyoko Kurosawa.

ten include immunosuppressed hosts (Bargellini et al., 2013). Finally, the incubation period may also be dose-dependent (Prasad et al., 2017).

Initial symptoms of Legionnaires' disease typically include fever, cough, and myalgias. Other commonly reported symptoms include headache, confusion, shortness of breath, sputum production, anorexia, nausea, and diarrhea; patients with community-acquired pneumonia who present with neurologic and gastrointestinal symptoms may be more likely to have legionellosis (CDC, 2017; Cunha, 1998). Some patients with *Legionella* pneumonia present with acute respiratory failure, hypotension, and sepsis-like signs that can mimic other common causes of bacterial pneumonia (e.g., *Streptococcus pneumoniae*). In contrast, patients who are immunosuppressed may present without fever, cough, or other more typical symptoms (del Castillo et al., 2016; Sivagnanam and Pergam, 2016).

Pontiac fever presentation is less specific and therefore less frequently reported. It is often described as a "flu-like" illness, with fever, headaches, and myalgias as the primary symptoms; chills, vertigo, diarrhea, and physical weakness or lack of energy (asthenia) are other symptoms (Tossa et al., 2006). Pontiac fever cases are defined in part by their absence of pneumonia. Since many other illnesses resemble Pontiac fever, the diagnosis usually relies on the recognition of typical clinical features during an outbreak situation; therefore, sporadic cases are likely to be missed (Murdoch, 2003). Pontiac fever in particular may be underdiagnosed in children, whose febrile illnesses are frequent and often self-limited (Qin et al., 2002).

It is unclear why patients develop Pontiac fever rather than pneumonia; consequently, the pathogenesis of the disease remains unclear. Several pathways for Pontiac fever have been hypothesized, including bacterial toxins, allergic responses, and exposure and reaction to *Legionella*-carrying ameobae (Edelstein, 2007). A self-limiting disease, Pontiac fever does not require treatment with antibotic therapy, leading some to hypothesize that the disease is not directly related to infection by these bacteria. At the same time, there have been outbreaks where patients who develop Pontiac fever have positive urinary antigen testing, suggesting that the disease is associated with ingestion or inhalation of either live or dead microorganisms (Burnsed, 2007). Mechanism of exposure may also play a role, as some recreational outbreaks have been linked to both *Legionella* pneumonia and Pontiac fever, whereas others are tied only to Pontiac fever (Euser et al., 2010; Leoni et al., 2018).

Not all *Legionella* spp. are thought to cause Pontiac fever, as the disease is most frequently linked to *L. pneumophila* exposures. Similar to pneumonia, however, non-*pneumophila* species such as *L. feelii, L. micdadei,* and *L. bozemanii* can also cause Pontiac fever (Cramp et al., 2010; Fentersheib et al., 1990; Fields et al., 2001; Herwaldt et al., 1984; Huhn et al., 2002). Only one study has suggested Pontaic fever was associated with exposure to water sources with higher concentrations of *Legionella* spp. (i.e., more than 10^3 colony forming untis per liter [CFU/L]) (Remen et al., 2011). Interestingly, in the same study, younger nursing staff were at higher risk, suggesting immune responses to prolonged or prior exposures may provide protection from this form of disease. Species-specific strains or particular bacterial activities themselves may be critical to disease outcomes. For example, a *L. fellii* serogroup with a monopolar flagellum associated with *Legionella* pneumonia showed a higher cell infection rate, stronger internalization by host cells, and greater cytotoxicity in vitro than a different *L. fellii* serogroup without a flagellum that was associated with Pontiac fever (Wang et al., 2015a). The lower risk associated with Pontiac fever, the limited diagnostic work-up, and the rarity of documented positive cultures linked to the disease make studies of pathogenesis difficult. Regardless of the outstanding questions concerning the pathogenesis of Pontiac fever, it is clear that exposure to water or soil contaminated with *Legionella* spp. is required for the development of the disease.

Diagnosis of Legionellosis

Presenting Laboratory Findings

Beyond clinical symptoms, laboratory findings may point to a diagnosis of *Legionella* pneumonia. Patients can present with either leukocytosis or leukopenia, hyponatremia, elevated liver enzymes, and renal dysfunction. Non-specific blood tests that suggest inflammation (e.g., C-reactive protein) can also be elevated (Fiumefreddo et al., 2009). Clinical prediction tools, such as the Winthrop-University Hospital criteria (Cunha et al., 1998), Community-Based Pneumonia Incidence Study Group score (Fernández-Sabé et al., 2003), Japan Respiratory Society score (Yanagihara et al., 2001) and a six-parameter clinical score developed by Fiumfreddo and colleagues (2009) are thought to have poor sensitivity for legionellosis, but may be useful for their negative predictive value (Miyashita et al., 2017).

Radiology

There are no unique radiologic findings specific for legionellosis. For *Legionella* pneumonia, chest radiographs often demonstrate focal infiltrates or consolidations consistent with pneumonia (Poirier et al., 2017). Computed tomography (CT) findings may show multi-lobar or air-space disease with associated ground-glass opacities; and lymphadenopathy and pleural effusions may be seen as well (Mittal et al., 2017). Among the immunocompromised, *Legionella* can present as pulmonary nodules with or without cavitation (del Castillo et al., 2016; Mittal et al., 2017). Patients with Pontiac fever are defined by their lack of findings on radiologic imaging.

Legionella-Specific Diagnostics

Culture. Cultures can be collected from pulmonary and extra-pulmonary sites (Mercante and Winchell, 2015). *Legionella* requires special culture media, most commonly buffered charcoal yeast extract agar (see Descours et al., 2014, for a recent analysis of different media). Growth usually occurs within three to five days, although two weeks may be required, as antibiotics used to reduce background respiratory microbiota can inhibit *Legionella* growth (Pierre et al., 2017). Growth from clinical samples may be limited or delayed if, prior to specimen collection, patients have been given antibiotics targeting *Legionella* spp. Non-*pneumophila Legionella* spp. tend to be more fastidious, may require longer incubation times (Mercante and Winchell, 2015), may be inhibited by some culture media (Lee et al., 1993), and require considerable technical laboratory expertise to culture (Lucas et al., 2011). Most culture-based systems are optimized for *L. pneumophila* and may limit growth of non-*pneumophila* species. Another complication arises when patients have multiple strains of *Legionella* during an active infection (Coscolla et al., 2014; Zhang et al., 2014). Because of these challenges, most hospital-based laboratories do not routinely test for *Legionella* spp. by culture. Yet, cultures are critically important to epidemiologic investigations, as they allow for analysis of relatedness within clusters and between clinical and environmental samples. In the United States culture diagnosis has declined from more than 60 percent in the early 1990s to 5 percent from 2005 to 2009 (Mercante and Winchell, 2015). In Europe, where cultures are more often utilized, 79 percent of culture-confirmed cases were reported as *L. pneumophila* serogroup 1 (ECDC, 2019).

Urinary Assay. The *Legionella* urinary antigen test (UAT) is the most frequently used method for legionellosis diagnosis in the United States (Mercante and Winchell, 2015). The UAT is routinely available on-site at 25 percent of acute-care hospitals (Garrison et al., 2014) and commercial laboratories. The test is popular because of the rapid turn-around time for on-site laboratories, ease of use, high sensitivity, and the ability to identify *L. pneumophila* serogroup 1, the most prevalent *Legionella* spp. associated with clinical disease, without the need for invasive procedures. However, the UAT can be negative very early in the disease and is of limited value in patients who cannot produce urine (anuric), e.g., due to kidney failure. On the other hand, the UAT can remain positive for months after an infection, particularly in immunosuppressed patient populations (Kashuba and Ballow, 1996; Kohler et al., 1984; Munoz et al., 2009). A major limitation of diagnosis strategies that focus on the UAT alone is their failure to detect important non-serogroup 1 *L. pneumophila* infections and non-*pneumophila* infections.

Serology. Acute and convalescent titers for *Legionella* may be helpful in documenting *Legionella* exposures, but have limited sensitivity for confirmation of Legionnaires' disease (Botelho-Nevers et al., 2016; Plouffe et al., 1995). Indeed, up to 20 to 30 percent of patients with proven *Legionella* may not mount an antibody response sufficient for diagnosis (Benz-Lemoine et al., 1991). For patients with altered immunity, the sensitivity and the specificity of seroconversion to non-*pneumophila Legionella* spp. is unclear (Reller et al., 2003). Even with the presense of high levels of antibody, one cannot differentiate recent versus past exposure, limiting the use of serology in acute infections (Mercante and Winchell, 2015). Serological testing for *Legionella* can also cross-react with *Coxiella burnetti* (the agent of Q fever) and *Mycoplasma pneumoniae*, among others (Boswell et al., 1996; Musso and Raoult, 1997).

Serology can provide important information for epidemiologic investigations. In a large outbreak in Norway, acute-phase *Legionella* tests (i.e., culture, UAT, and PCR) detected about 56 cases, whereas serology detected an additional 47 cases (Simonsen et al., 2015). Thus, serology may identify individuals with less severe disease and symptoms (e.g., Pontiac fever) who might otherwise be missed during large industrial exposures and outbreaks. Nevertheless, the use of serology diagnosis for either sporadic disease or outbreak investigation has declined (Mercante and Winchell, 2015).

Direct Fluorescent Antibody Testing. Direct fluorescent antibody (DFA) testing is infrequently used to diagnose *Legionella*. The sensitivity of DFA can be very low (11 to 40 percent) (Hayden et al., 2001; She et al., 2007), and it often does not detect non-*pneumophila Legionella* spp. (Reller et al., 2003). Although some argue DFA has high specificity when positive, caution is needed as a positive DFA in the absence of other supportive evidence is thought to be insufficient for *Legionella* diagnosis (Haldane et al., 1993; Reller et al., 2003).

Molecular Testing. Polymerase chain reaction (PCR) and other nucleic acid amplification tests are highly sensitive assays for lower respiratory tract specimens but are primarily available in referral or research laboratories. Most published studies utilize PCR testing that targets the gene encoding the macrophage infectivity potentiator (*mip*) surface protein of *L. pneumophila* (Phin et al., 2014). One study found a four-fold increase in *Legionella* case detection with PCR testing of lower-respiratory specimens compared to culture (Murdoch et al., 2013), and another found only 40 percent of PCR-positive specimens were also culture-positive (Rucinski et al., 2018). Compared to UAT, PCR tests may be more sensitive, detecting an additional 18 to 30 percent of cases (Avni et al., 2016). PCR may have the advantage for *Legionella* diagnosis in patients already on empiric therapy with *Legionella* active antibiotics, which limit bacterial growth by culture.

Most PCR methods currently detect serogroup 1 strains but cannot distinguish among *L. pneumophila* serogroups (Benitez and Winchell, 2013), and most probes target *L. pneumophila* specifically. There are newer PCR assays that target common non-*pneumophila* species including *L. longbeachae, L. micdadei,* and others (Cross et al., 2016). Broader *Legionella* spp. PCR tests have been developed (Benitez and Winchell, 2013; Chen et al., 2015), but to date only one test has been approved by the U.S. Food and Drug Administration (FDA) for testing of clinical samples. The BioFire® FilmArray® Pneumonia Panel is a multiplex PCR respiratory panel that detects 33 different respiratory pathogens, including *L. pneumophila*. The assay was approved in November 2018 for sputum, endotracheal aspirates, and bronchoalveolar lavage (or mini-BAL) lower-respiratory tract samples. PCR assays have been licensed in Europe, but most available assays are primarily laboratory-developed assays of variable sensitivity (Ricci et al., 2018). Of note, PCR is less sensitive for non-respiratory samples (e.g., blood; Avni et al., 2016; von Baum et al., 2008).

Pharmaceutical Therapy

Legionella spp. are intracellular pathogens that not only avoid phagosome–lysosome fusion and degradation, but also replicate within alveolar macrophages and epithelial cells (Newton et al., 2010). Therefore, the mainstay of drug therapy for legionellosis are antibiotics that target the intracellular space. Guidelines in the United States and in Europe recommend macrolides and fluoroquinolones as first-line therapy (Mandel et al., 2007; Pea, 2018; Woodhead et al., 2011). Macrolides work primarily by disrupting the 50S subunit of bacterial ribosomes, thereby inhibiting protein synthesis, which is critical for the microbe's survival. Most early studies evaluated the macrolides erythromycin and clarithromycin, but azithromycin is the preferred agent as it is better tolerated (Langley et al., 2004), associated with fewer side effects than other macrolides, and is the most effective macrolide in animal models (Fitzgeorge et al., 1990; Plouffe et al., 2003).

Compared with macrolides, fluoroquinolones, which inhibit bacterial DNA gyrase and topoisomerase IV enzymes, are more potent against *Legionella* spp. in both in vitro and in vivo models of infection (Pedro-Botet and Yu, 2009). Currently, there is no randomized clinical trial comparing macrolides to

fluoroquinolones for antibiotic therapy of legionellosis. However, in non-randomized, observational studies, fluoroquinolones were more effective than macrolides (i.e., erythromycin and clarithromycin) in fever resolution and length of hospitalization (Burdet et al., 2014; Garcia-Vidal et al., 2017; Griffin et al., 2010). Despite therapy, mortality rates for cases treated with both drug classes remain around 10 percent (Burdet et al., 2014).

Beyond macrolides and fluoroquinolones, other agents such as rifampin/rifampicin, tetracyclines, and trimethoprim-sulfa are used, sometimes in combination (Pedro-Botet and Yu, 2009). Although data are limited, some experts recommend that patients with severe clinical illness may benefit from combination therapy (Nakamura et al., 2009; Varner et al., 2011).

Selection for antibiotic-resistant *Legionella* is considered very limited, as humans are a dead end in the life cycle of *Legionella* spp. Indeed, just one case of suspected person-to-person tranmission has been reported in more than 40 years (Correia et al., 2016). Although infrequent, there are data demonstrating both clinical failures and *Legionella* that, under macrolide pressure, have documented macrolide resistance primarily through mutations in the bacterial ribosome (Descours et al., 2017; Dowling et al., 1985; Nielsen et al., 2000). To date, fully resistant strains have not been observed in wild-type isolates (Vandewalle-Capo et al., 2017). There is also concern that widespread use of azithromycin for other conditions among hospitalized patients could lead to increased resistance in *Legionella* (Torre et al., 2018). Similar to macrolides, rare resistance to fluoroquinolones has been identified, primarily through changes in bacterial DNA gryrase genes (Almahmoud et al., 2009; Bruin et al., 2014; Jonas et al., 2003).

LEGIONELLA SPECIES AND STRAINS

The *Legionella* genus is in constant flux, blurring the boundaries between species. Variation is generated by high rates of genetic exchange among species as well as by homologous recombination between exogenous DNA and the bacterial chromosome (Gomez-Valero et al., 2014, 2019; Joseph et al., 2016; Sanchez-Buso, 2014). Within the genus, both genome size and guanine-cytosine (GC) content vary widely (see Figure 2-3). Furthermore, among the pangenome of 80 sequenced *Legionella* strains representing 58 species, only 6 percent of the genes were encoded by each genome examined (Gomez-Valero et al., 2019). For example, although the flagellar regulon contributes to *L. pneumophila* infection of macrophages and amoebae (reviewed by Appelt and Heuner, 2017), flagella genes were absent in 23 of 80 sequenced genomes from 58 *Legionella* spp. (Gomez-Valero et al., 2019).

To further guide diagnosis and identification of the environmental source of the infectious bacteria, *L. pneumophila* strains can be classified by several techniques. Terms commonly used to describe particular isolates of bacteria are explained in Figure 2-4.

L. pneumophila can be divided into serogroups according to the structure of its lipopolysaccharide (LPS), a component of the outer membrane of these Gram-negative bacteria and the predominant antigen recognized by the human immune system (Ciesielski et al., 1986; Conlan and Ashworth, 1986). Although 16 distinct "serogroups" of *L. pneumophila* have been identified, the majority of disease cases are caused by serogroup 1 strains. For example, in Europe from 2011 to 2015, serogroup 1 strains were associated with 83 percent of the cases confirmed by culture (Beauté, 2017), a frequency similar to that reported by a 2002 international prospective study (Yu et al., 2002). More rarely, strains of other serogroups are isolated from Legionnaires' disease patients, and the pattern varies by region. In two international studies, serogroups 3, 4, 5, 6, and 10 were each associated with less than 2 percent of culture-confirmed cases (Beauté, 2017; Yu et al., 2002). In Denmark from 1993 to 2006, serogroup 3 was identified in 23 percent and serogroup 6 in 5 percent of 419 culture-confirmed cases (St. Martin et al., 2013). In contrast,

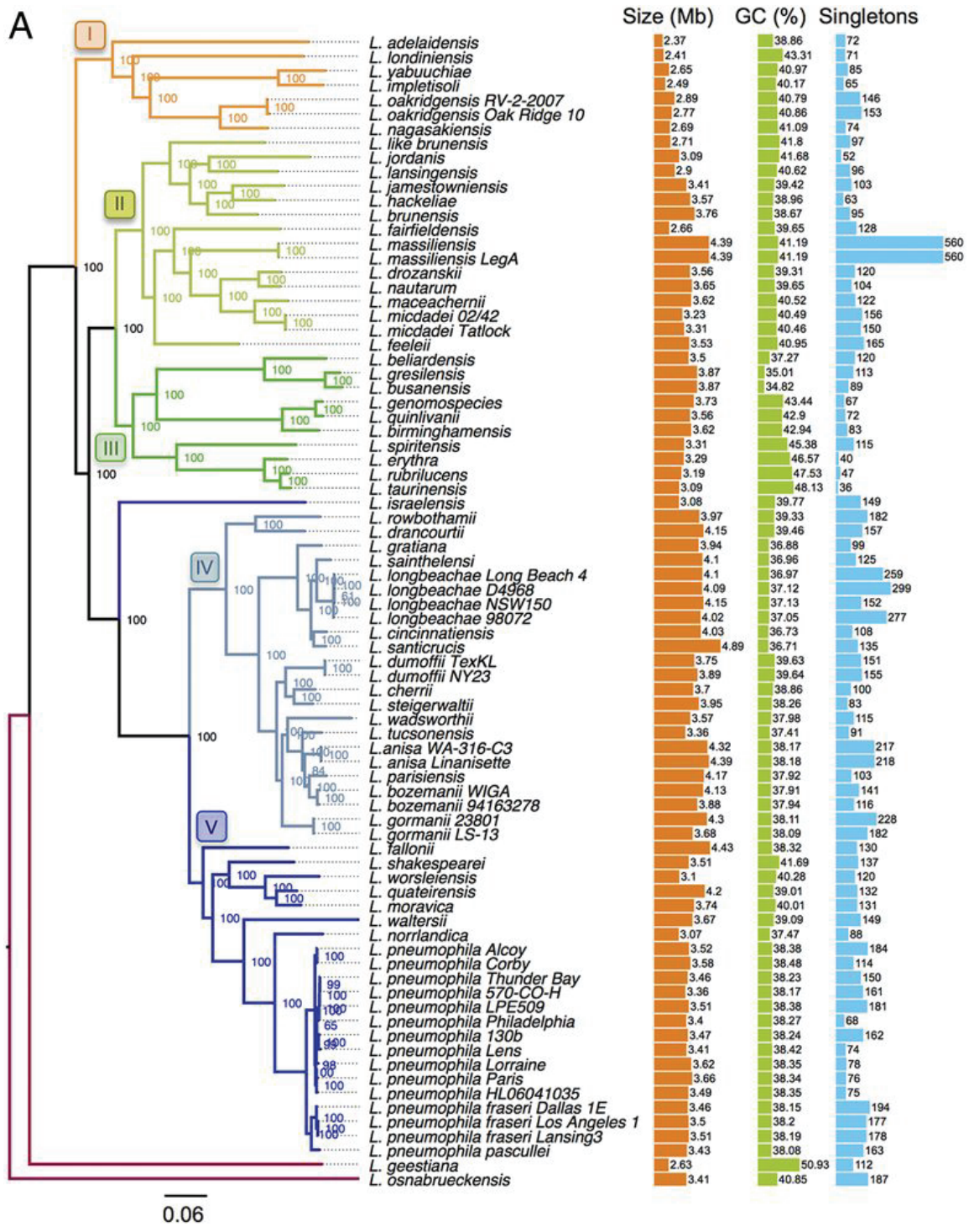

FIGURE 2-3 Diversity of size and gene content of *Legionella* genomes. Phylogeny of the genus based on the core genome, genome size, GC content, and number of singletons (genes identified in only a single species) of each species are depicted. Numbers represent bootstrap values. Branches are colored according to the clade (lineages that share a common ancestor) to which they belong. Genome size and GC content include plasmids if present in the corresponding species. The number of singletons is based on the results of OrthoMCL (takes into account orthologs and paralogs). Each species has been compared with the others without taking into account strains from the same species to avoid bias due to the number of strains sequenced within a species. SOURCE: Gomez-Valero et al. (2019).

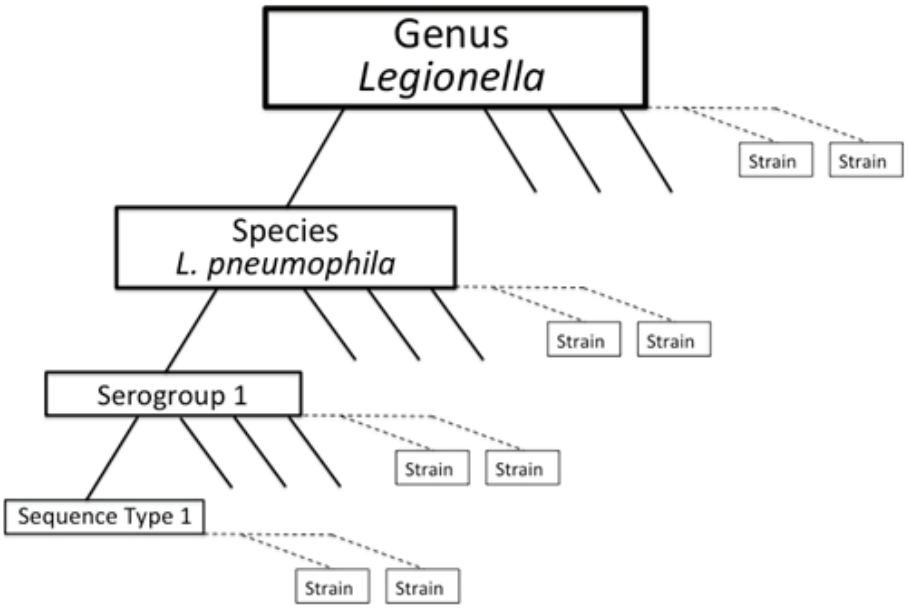

FIGURE 2-4 Nomenclature of *Legionella*. Genus and species are taxonomic categories that denote the degree of similarity among organisms. Serogroup refers to a distinct antigenic profile of the outer membrane lipopolysaccharide. Sequence type is defined by the DNA sequence of seven genomic loci designated by the field. Strain (dashed lines) is a more general term used to distinguish bacterial isolates without reference to their genetic or antigenic identity; for example, *L. pneumophila* cultures isolated independently are initially given different strain names until additional information on species, serogroup, or sequence type is obtained.

in Ontario, Canada, serogroup 6 *L. pneumophila* accounted for 47 percent of the disease cases not caused by serogroup 1 strains (Khan et al., 2013). In Michigan, serogroup 6 *L. pneumophila* also predominated in a surveillance study of premise plumbing one year after the 2014-2015 Legionnaires' disease outbreaks in Flint (Byrne et al., 2018).

L. pneumophila is the most prevalent reported species of *Legionella* in building water systems, whereas non-*pneumophila* strains show more geographic variation. Recent surveillance studies of hundreds of buildings in Germany and Hungary identified 58 to 84 percent of the isolates as *L. pneumophila* (Barna et al., 2016; Dilger et al., 2018; Kruse et al., 2016). Temperature is likely one factor that influences the prevalence of particular species. For example, in a study of more than 13,000 warm-water systems in southern Germany, *L. anisa* composed 18 percent of the total isolates obtained from water at 20°C but only 8 percent at 60°C (Dilger et al., 2018).

The clinical predominance of *L. pneumophila* serogroup 1 does not simply reflect a relative abundance in the environment (Doleans et al., 2004; reviewed by Mercante and Winchell, 2015). It also appears to grow better in humans than other *Legionella* species. For example, compared with seven other serogroups, the composition of serogroup 1 LPS equips *L. pneumophila* to resist killing by the alternate complement pathway (Khan et al., 2013), a key barrier of the human innate immune system. The serogroup 1 LPS O-antigen is also extremely hydrophobic (Zähringer et al., 1995), which may contribute to *L. pneumophila* survival in the human lung via evasion of toxic lysosomes (Fernandez-Moreira et al., 2006) or perhaps within aerosols. Two other observations suggest that the serogroup 1 LPS enhances

L. pneumophila virulence. Unlike environmental strains, the majority of clinical isolates display a distinct LPS epitope encoded by the *lag-1* gene and recognized by particular monoclonal antibodies (MAb2 and MAb3/1), a structural feature that is used for diagnosis and risk-assessment (Kozak et al., 2009). It is also striking that the only genes shared by greater than 200 serogroup 1 strains comprise a locus dedicated to LPS biosynthesis (Cazalet et al., 2008). A cluster of these serogroup 1 LPS genes can spread horizontally among the legionellae (Cazalet et al., 2008; Merault et al., 2011) expanding the genetic diversity of serogroup 1 strains. Moreover, the LPS locus of at least two *L. pneumophila* serogroup 1 lineages exhibits an elevated rate of genetic exchange (David et al., 2017), further expanding their diversity. Whether serogroup 1 strains are better equipped to survive in aerosols or to colonize engineered water systems warrants investigation.

Because the *L. pneumophila* genome is dynamic, related strains of the same serogroup must be distinguished using molecular methods. Since 2005, the international *Legionella* community has applied a multi-locus DNA sequence typing scheme and online tools (Gaia et al., 2005; Ratzow et al., 2007; Underwood et al., 2006). Currently, more than 2,000 *L. pneumophila* sequence types (STs) can be distinguished based on the nucleotide sequence of seven alleles. By combining sequence-based typing with classical epidemiology, investigators can perform trace-back studies to identify outbreak sources, as well as assess regional patterns and persistence in particular environments. For example, Kozak-Muizniek and colleagues (2016) conducted a comprehensive phylogenetic analysis of hundreds of *L. pneumophila* strains collected in the United States from outbreaks, sporadic clinical cases, and environmental surveillance, all of which were submitted to the CDC over a 30-year period.

Worldwide, the most prevalent sequence type is ST1 within serogroup 1. In the United States from 1982 to 2012, this sequence type accounted for 49 percent of the *L. pneumophila* strains obtained from water and 25 percent of the sporadic disease isolates (Kozak-Muizniek et al., 2016). Likewise, in China over a seven-year period, 49 percent of environmental isolates were ST1 *L. pneumophila* (Qin et al., 2014). Multi-year surveillance studies in England, France, and Wales identified ST1 *L. pneumophila* as composing approximately 20 percent of environmental strains (Cassier et al., 2015; Harrison et al., 2009), and a Japanese analysis discovered that 74 percent of *L. pneumophila* isolates from cooling towers were ST1 (Amemura-Maekawa et al., 2012). Nevertheless, even among ST1 serogroup 1 *L. pneumophila* genomes, substantial diversity is generated by genetic recombination, as evident from whole genome sequence analysis of more than 200 ST1 strains (David et al., 2017). Thus sequence-typing alone is not sufficient to establish epidemiological associations of ST1 strains.

Nonetheless, sequence-based typing of large strain collections has identified regional variations in the endemic *L. pneumophila* populations and infection patterns. For example, ST1 strains caused multiple outbreaks in Belgium from 2000 to 2010 (Vekens et al., 2012), but the first outbreak in the United States was not recorded until 2012 (Kozak-Muiznieks et al., 2014). And although ST222 strains of *L. pneumophila* appear to be endemic in the northeastern regions of the United States and Canada, this type has been reported only recently and just once in three other countries (Byrne et al., 2018; Kozak et al., 2009; Kozak-Muiznieks et al., 2016; Tijet et al., 2010). In Western Europe, the *L. pneumophila* strain type most frequently isolated from patients is ST47 (also known as the Lorraine strain), yet no Asian or U.S. case has been attributed to this genotype (reviewed by Kozak-Muizniek et al., 2016). The biological basis for either the geographic distribution or the clinical prevalence of particular strains of legionellae remains to be determined. Knowledge of the genetic attributes that increase *L. pneumophila* fitness in distinct environments could guide detection and remediation strategies for particular geographic regions.

Despite the diversity of legionellae, research on the virulence and resilience mechanisms is focused on *L. pneumophila* serogroup 1 strains. Indeed, a literature search for titles and abstracts that contain "pneumophila" and "serogroup 1" identified more than 450 research articles; in contrast, "pneu-

mophila" and "serogroup 6" identified just 64. Even more striking, a title search for "pneumophila" identified approximately 3,000 articles, whereas "micdadei" returned only 101. With the rapidly expanding opportunities to integrate epidemiological studies, genomics, and laboratory-based tests of virulence and persistence, the field is poised to advance knowledge of how, when, and where these common aquatic and soil microbes threaten human health.

L. pneumophila Life Cycle

As an environmental microbe, *L. pneumophila* adapts to fluctuating conditions by altering its physiology. Multiple distinct cell types have been defined based on a combination of morphological, biochemical, genetic, and molecular features (see Box 2-1; Robertson et al., 2014). Depending on its environment, *L. pneumophila* can differentiate between replicative, transmissive, filamentous, mature infectious forms, and viable-but-not-culturable-like (VBNC-like) cells (see Figure 2-5). *L. pneumophila* cells obtained from solid media are a mix of replicating, transmissive, and filamentous cell types, whereas more homogenous cell populations can be isolated from broth cultures. Each specialized cell type differs in its capacity to infect host cells and tolerate antibiotics, biocides, and other environmental stresses, as discussed below.

Replicative and transmissive forms are two cell types observed when culturing *L. pneumophila* in rich bacteriological media at 37°C with aeration. Replicative cells are isolated during the exponential growth phase, and the transmissive cell type is generated in the stationary phase (Brüggemann et al., 2006; Byrne and Swanson, 1998). To alternate between replicative and transmissive states, *L. pneumophila*

BOX 2-1
Bacterial Cell Terminology

Culturable: Bacteria that can be grown on a suitable culture medium.

Infectious: Bacteria capable of entering, surviving, and replicating within host cells or tissues.

Mature infectious form: A *L. pneumophila* a cell type that develops efficiently within certain host cells, contains abundant energy storage inclusions, and, compared to transmissive cells, has thicker outer membranes and is even more infectious and resistant to a variety of stresses, including certain antibiotics, detergents, and basic pH.

Replicative: A cell type that is actively multiplying or in an exponential growth phase. When in this state, *L. pneumophila* expresses pathways needed to generate progeny cells but not factors that promote infection or its spread from one host cell to another nor resistance to a variety of stresses.

Transmissive: A cell type that is not replicating (post-exponential or stationary phase) but is motile, spreads among host cells, and evades host lysosomes. Compared to replicative cells, transmissive cells are more resistant to a variety of stresses, including heat, osmotic pressure, and acidic pH.

Viable: Bacteria that are metabolically active and retain the capacity to grow when conditions are suitable.

Viable but non-culturable: A reversible dormant state in which bacteria have intact outer membranes and are metabolically active, yet do not grow on suitable media.

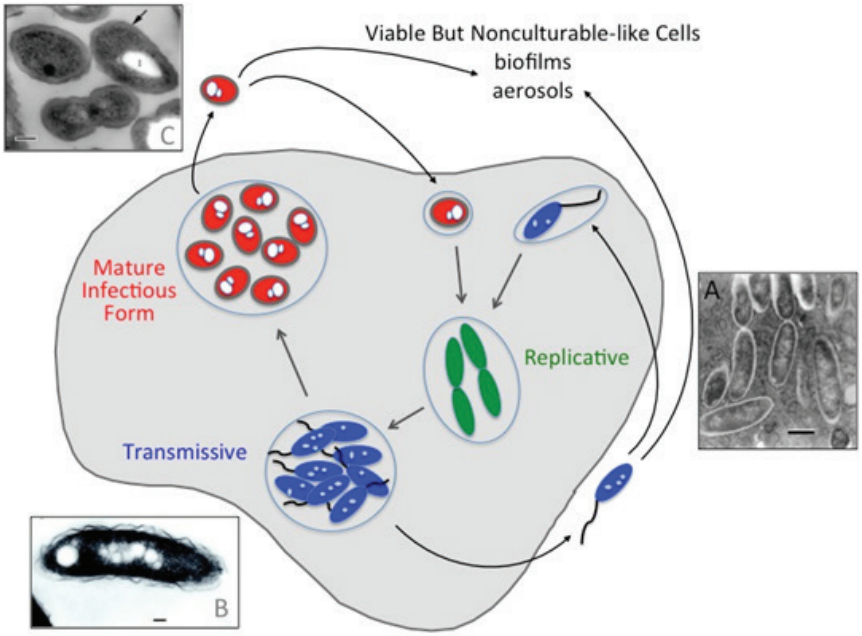

FIGURE 2-5 *L. pneumophila* alternates between multiple cell types within a host protozoan (gray). (A) replicative cells lack cytoplasmic inclusions; scale bar = 0.5 μm. (B) transmissive or stationary phase cells have wavy outer membranes, pseudomembranous inclusions, and polar flagella; scale bar = 0.1 μm. (C) mature infectious form cells have thickened outer membranes (arrow) and large inclusions (I); scale bar = 100 nm or 0.1 μm. Once released from the host, *L. pneumophila* may differentiate to a VBNC-like form, attach to biofilm, or be spread in aerosols.
SOURCES: Faulkner et al. (2008); Faulkner and Garduño (2002); Garduño et al. (2002).

reprograms its gene expression and metabolic profile (Brüggemann et al., 2006; Dalebroux et al., 2009; Faucher et al., 2011, reviewed by Oliva et al., 2018). For example, when nutrients are plentiful in broth, amoebae, or macrophages, *L. pneumophila* rely on the regulatory protein CsrA to repress production of not only flagella but also virulence factors that promote transmission between host cells, while activating pathways that catabolize serine and glucose and generate energy storage granules, or "inclusions" of polyhydroxybutyrate (PHB; Eylert et al., 2010; Gillmaier et al., 2016; Häuslein et al., 2016, 2017; James et al., 1999; Sahr et al., 2017). Once nutrients become limiting, *CsrA* repression is relieved, and *L. pneumophila* begin to catabolize PHB, utilize glycerol as a precursor for biosynthesis, modify their LPS and surface composition, acquire stress resistance, assemble a flagella, produce numerous virulence factors for export by a Type IV secretion system and become competent to evade phagosome-lysosome fusion (Eylert et al., 2010; Fernandez-Moreira et al., 2006; Harada et al., 2010; Häuslein et al., 2016, 2017; Mendis et al., 2018; Nevo et al., 2014; Sahr et al., 2017; Trigui et al., 2015). Thus, replicative phase cells specialize in generating progeny, whereas transmissive cells are primed to escape one host cell, survive and disperse in the environment, and establish a protected replication niche in a new host cell.

In water and in lungs, a filamentous cell type of *L. pneumophila* has been observed (reviewed by Robertson et al., 2014). In general, filamentation is observed after exposure to environmental stress, including scarce nutrients, high temperatures, ultraviolet (UV) light, or antibiotics. Relatively few studies have focused on filamentous forms, and the regulatory controls that govern filamentation are not known. Nevertheless, some fitness advantages have been described. When *L. pneumophila* are cultured without aeration in rich media at 37°C, their elongated morphology likely enhances attachment to biofilm, as thick meshworks of multinucleate, filamentous cells readily adhere to glass (Piao et al., 2006).

When co-cultured with epithelial cells and macrophages, filamentous *L. pneumophila* are internalized within phagolysosomal compartments that fail to seal; thus, these leaky host vacuoles are less toxic to the elongated prey (Prashar et al., 2012, 2013). Filamentous cells can revert to the typical short rod morphology once static cultures are aerated (Piao et al., 2006) or after ingestion by macrophages (Prashar et al., 2013). Additional research is needed to illuminate the molecular mechanisms that govern filamentation and when and where this multinucleate elongated cell type increases *L. pneumophila* fitness.

In amoebae and ciliates, intracellular *L. pneumophila* can further differentiate into hardy mature infectious forms (MIF; Faulkner and Garduño, 2002; reviewed by Robertson et al., 2014). Such MIF cells are characterized by thick cell walls, abundant PHB storage granules, and metabolic dormancy. Compared with cells obtained from either stationary phase broth or macrophage cultures, MIF *L. pneumophila* are more resistant, by orders of magnitude, to basic pH, detergents, chlorine, and antibiotics, and they adhere to macrophages more readily (Abdelhady and Garduño, 2013). MIFs can also be released from amoebae and ciliates within membrane-bounded vesicles, which provide an additional layer of protection. Thus, MIF cells appear to be well equipped for transmission within aerosols to the human lung (Robertson et al., 2014).

Once either transmissive or MIF *L. pneumophila* encounter adequate nutrients within host amoebae or macrophages, they return to a replicative form that generates numerous progeny. For example, transmissive *L. pneumophila* will persist as single cells within macrophages or amoebae vacuoles until adequate threonine is acquired; subsequently, the bacteria switch to the replicative form (Sauer et al., 2005). Within amoebae, robust *L. pneumophila* replication ensues when arginine relieves transcriptional repression by the arginine repressor, ArgR (Hovel-Miner et al., 2009, 2010). Transporter proteins embedded in the host vacuolar and bacterial membranes equip replicative *L. pneumophila* to obtain amino acids needed for bacterial replication (Schunder et al., 2014).

After prolonged environmental stress, *L. pneumophila* is thought to differentiate into a VBNC-like cell type. Described for many bacteria, such a VBNC adaptation increases bacterial resistance but also impedes environmental surveillance strategies that rely on culture (reviewed by Li et al., 2014). *Vibrio vulnificus* is a marine Gram-negative pathogen whose VBNC state has been analyzed in molecular detail. After four days exposure to 5°C artificial seawater, culturability of *V. vulnificus* declines 8 logs. Once the cell suspension is returned to 22°C, within less than 10 hours culturability rapidly increases, by greater than 6 logs (Whitesides and Oliver, 1997). This efficient revival is coordinated by a quorum sensing signal transduction system (Ayrapetyan et al., 2014). Thus, the aquatic pathogen *V. vulnificus* is genetically programmed to respond to shifts in temperature and cell density by efficiently alternating between replication-competent and VBNC states.

Compared to *V. vulnificus,* the capacity of *Legionella* spp. to alternate between replication and a resilient VBNC-like state remains enigmatic. When MIF *L. pneumophila* are held for weeks in hot, nutrient-poor water, the majority acquire features of VBNC cells (Al-Bana et al., 2014). For example, 30 days after exposure to 45°C double-deionized water, the capacity of MIFs to form colonies on rich media steadily declines by more than 6 logs; yet, more than 80 percent of the cells have an intact cell membrane, as judged by cell membrane integrity stains. These MIF-derived, VBNC-like cells are resistant to detergent and remain intact when co-cultured with the ciliate hosts *Tetrahymena tropicalis* and *T. thermophila*, as judged by qualitative electron microscopy studies. However, MIF-derived, VBNC-like bacteria do not readily resume replication when cultured on rich medium or when ingested by a range of host phagocytes known to support robust *L. pneumophila* replication, including *Acanthamoebae castellanii, T. tropicalis, T. thermophila,* and human monocytic mouse fibroblast cell lines (Al-Bana et al., 2014). Therefore, whether MIF-derived, VBNC-like *L. pneumophila* pose a risk to public health remains an open question.

Compared to MIF cells, transmissive *L. pneumophila* exhibit a different profile after prolonged exposure to 45°C tap water (Al-Bana et al., 2014). After 45 days, culturability declines more than 8 logs, but only about 20 percent of the population maintains viability, as judged by either vital staining or qualitative ultrastructure analysis. Although these VBNC-like cells remain intact after treatment with detergent or ingestion by *A. castellani,* only about 1 in 10^5 of this population resumes replication in this permissive amoebae host.

Several investigations have reported that nutrient, chemical, or temperature stress triggers *L. pneumophila* to differentiate into a VBNC-like cell type (Alleron et al., 2008; Dietersdorfer et al., 2018; Ducret et al., 2014; García et al., 2007; Hwang et al., 2006; Kirschner, 2016; Schrammel et al., 2018). In general, after prolonged stress, stationary phase *L. pneumophila* lose culturability, yet bind a vital stain or retain intact ribosomes (Dietersdorfer et al., 2018; Epalle et al., 2015; Ohno et al., 2003; Steinert et al., 1997). Numerous experimental factors alter generation and resuscitation of these VBNC-like *L. pneumophila*, including water composition, bacterial strain, growth phase, cell density, and host cell type. Quantification of VBNC-like populations is also affected by the method used to prepare control dead cells, the medium in which cells are suspended, the density of the cell suspension, and the sensitivity of instruments used to detect fluorescence markers that distinguish between live and dead cells (Braun et al., 2019). In contrast to our mechanistic understanding of VBNC *V. vulnificus* and several other bacteria, neither the environmental conditions nor the regulatory pathways that stimulate *L. pneumophila* to alternate efficiently between VBNC-like and replicative or MIF cell types have been delineated.

A major outstanding question is whether VBNC-like *L. pneumophila* cause infections in humans. In early studies that utilized a guinea pig model to assess virulence of VBNC-like *L. pneumophila*, no viable bacteria were recovered after infection (Steinert et al., 1997). When co-cultured with amoebae, some VBNC-like cells appear to resume replication; however, the quantitative data provided make it difficult to rule out that a minor population of culturable cells survived the initial stress treatment and subsequently initiated the amoebae infection. In a recent comprehensive study, VBNC-like *L. pneumophila* obtained 221 days after starvation were 20- to 100-fold less infectious for human monocyte-derived macrophages than bacteria obtained from broth cultures (Dietersdorfer et al., 2018). Thus, more research is required to determine whether, or under what conditions, VBNC-like *L. pneumophila* can establish infections in humans.

A limitation of current laboratory research practices and disinfection studies is the failure to take into account the distinct cell types of *L. pneumophila*. Environmental surveillance largely relies on culture-based detection (ISO, 1998), which does not detect VBNC-like cells. Genomic DNA tests applied in the clinic or in the field cannot distinguish among dead, live, VBNC, or infectious bacteria. Sensitivity to detergents, biocides, antibiotics and other stressors differs dramatically for replicative, transmissive, and MIF cells (Abdelhady and Garduño, 2013; reviewed by Robertson et al., 2014). The capacity of specialized *L. pneumophila* cell types to survive in aerosols and consequently gain access to the human lung has not been analyzed. The biochemical and environmental conditions that trigger development or resuscitation of VBNC-like *L. pneumophila* are not yet understood. Therefore, development and application of a standardized set of molecular markers specific to each cell type would advance the *L. pneumophila* field and ultimately guide clinical treatment and environmental remediation practices. Markers are currently available for replicative and transmissive forms of *Legionella* (Sauer et al., 2005) and for MIF (Abdelhady and Garduño, 2013), but not for VBNC-like *Legionella* or *Legionella* residing in biofilms. In the meantime, it is imperative that clinical, epidemiological, and research investigators be cognizant of the *L. pneumophila* life cycle and move toward newer approaches that can identify the cell type(s) in their samples.

MICROBIAL ECOLOGY OF *LEGIONELLA*

The microbial ecology of legionellae has been poorly studied since *L. pneumophila* was first cultivated from the environment. Although the genus *Legionella* contains 61 species and DNA has been identified for several addditional *Legionella* spp. that have not yet been cultivated (Edagawa et al., 2008; Parthuisot et al., 2010; Wery et al., 2008; Wullings and van der Kooij, 2006), what is known about legionellae ecology is almost exclusively based on studies with *L. pneumophila.* The original isolation and identification of *L. pneumophila* from water environments and environmental aerosols led to the biased consideration of this pathogen as a planktonic aquatic bacterium. While it is certainly present in water environments, current understanding points to its growth along with other pathogenic legionellae within various free-living protozoan hosts that feed on bacteria associated with biofilms (i.e., surface-attached microbes and their extracellular matrix) (Hilbi et al., 2011). Indeed, several *Legionella* spp. are thought to have developed various virulence factors as defense against predation by free-living protozoa (i.e., protozoa that grow in the environment on natural organic matter and microbes, not obligate parasites that only grow within another living organism) (Cianciotto, 2015), as have various other genera of so-called amoeba-resisting bacterial pathogens (Kebbi-Beghdaji and Greub, 2014). This section discusses principles of *Legionella* ecology as well as the two growth habitats for pathogenic legionellae—its primary reservoir in nature and its secondary habitat in engineered environments that may generate infectious doses delivered via aerosols to humans.

Principles of *Legionella* Ecology

Biofilms consist of microorganisms that grow attached to moist soil, sediment, decaying organic matter or other solid surfaces. They are largely hydrated gels composed of extracellular polymeric substances, consisting of carbohydrates, fats, protein and nucleic acids excreted by bacteria (Flemming et al., 2016). Gradients of nutrients, pH, and oxygen within the biofilm matrix support the varying needs of different microorganisms in the heterogeneous biofilm community. Various protozoa, and later on microinvertebrates, will naturally develop and feed on biofilms, further influencing the microbial diversity of mature biofilms. In oligotrophic environments such as drinking water, a "mature" biofilm consisting of a relatively stable microbial community composition can take several years to develop (Martiny et al., 2003). In contrast, most published biofilm experiments are typically undertaken after a limited period of development (Storey et al., 2004a, 2011).

L. pneumophila may form a mono-species biofilm in the laboratory and grow necrophilically on other decaying cells (Temmerman et al., 2006). Moreover, free, inactive *Legionella* persist within biofilms (Hindré et al., 2008). However, **the primary growth habitat of *L. pneumophila* is within amoebae** (Kuiper et al., 2004) **or other free-living protozoa that are associated with biofilms** (Buse et al., 2012; Hellinga et al., 2015). Within these protozoan hosts associated with fixed or free-floating biofilms (Hsu et al., 2011), pathogenic legionellae can replicate to problematic levels (Ashbolt, 2015; Declerck et al., 2010; Hamilton et al., 2019).

As illustrated in Figure 2-6 and Figure 2-7, large numbers of *L. pneumophila* can be released from the biofilm environment within fragments of biofilm, within protozoan trophozoites and cysts, or within expelled amoebal vesicles (membrane-bound structures containing undigested food and microorganims). These released *L. pneumophila* cells then disperse in the bulk water phase among other planktonic microorganisms. The dependency of *L. pneumophila* growth on protozoan hosts that graze on biofilms associates the ecology of *L. pneumophila* indirectly to the ecology of its host protozoan and supporting biofilm microorganisms.

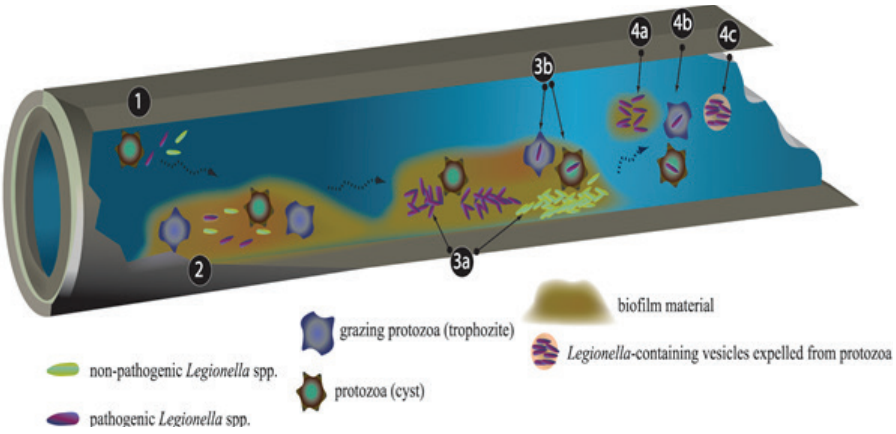

FIGURE 2-6 *Legionella* spp. along with various protozoa species in building water systems (1) and their associated biofilms (2), which contain a variety of microbes including bacteria, fungi, and higher organisms such as amoebae (not all of which are shown). *Legionella* either colonize the biofilm (3a) or are ingested by grazing protozoa (3b). The intracellular fate of *Legionella* after ingestion can vary: they are either digested by the protozoa, they can parasitize and eventually kill the protozoan host, or the protozoa may expel them in vesicles or the *Legionella* may persist within protozoan cysts. *Legionella* are then released from the biofilm in a variety of ways. *Legionella* bacteria that have colonized and proliferated within the biofilm can be released as this material sloughs off (4a), they can be found either within the trophozoite or cyst form of certain protozoa (4b), or they can be released within a vesicle derived from their protozoan host (4c). SOURCE: Lau and Ashbolt (2009).

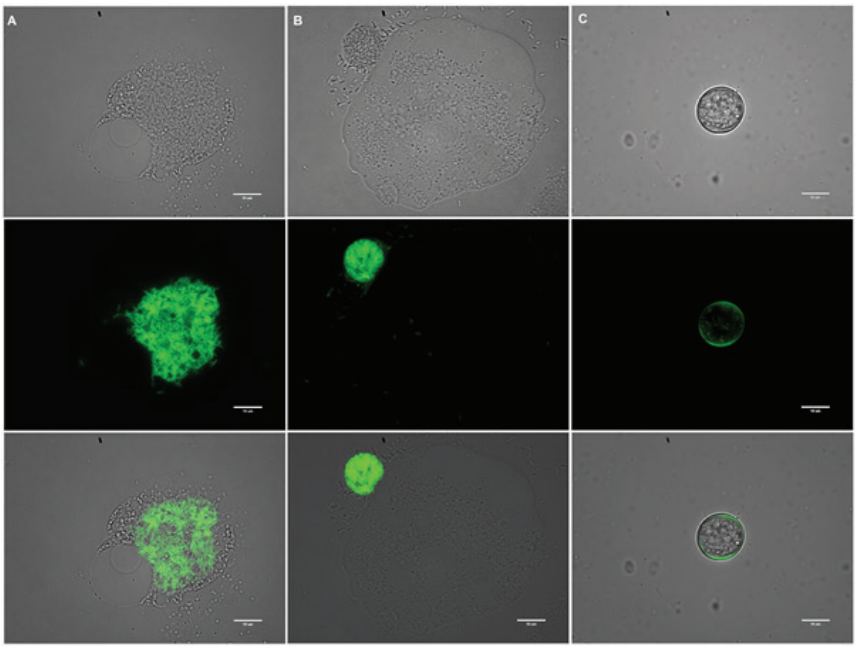

FIGURE 2-7 Micrographs of *Legionella* inside a protozoan host. The top row is light microscopy, the second row is fluorescence to highlight the viable *L. pneumophila* (expressing the green fluorescent protein gene) and the bottom row shows the top two rows combined. Panel A shows intracellular growth of *L. pneumophila* in Willaertia magna (ATCC 50035). Panel B shows the release of a vesicle containing *L. pneumophila* from W. magna (ATCC 50035) during intracellular growth. Panel C shows trapped *L. pneumophila* cells within a W. magna (ATCC 50035) cyst. SOURCE: Courtesy of Mohamed Shaheen.

Role of Free-Living Protozoa in Growth of Legionellae

Rowbotham (1980) first showed that *L. pneumophila* multiply in different protozoan hosts. Moreover, *L. pneumophila* multipled in sterile water when amoebae were added, but not when amoebae were removed by filtration (Kuiper et al., 2004; Nahapetian et al., 1991; Wadowsky et al., 1988). Subsequently, many free-living protozoa (amoebae and ciliates) have been identified that can serve as hosts for growth of *Legionella* (see Table 2-1). It is likely that other higher animal organisms can also serve as growth and/or transport hosts for *L. pneumophila* (Hellinga et al., 2015). Of note, *L. pneumophila* cells will not grow within the dry, dormant cysts of protozoa, although the bacteria are preserved and show increased resistance to disinfection proceses such as heat, chemicals, sonication, and UV (Cervero-Arago et al., 2014; Declerck et al., 2010; Storey et al., 2004b).

TABLE 2-1 Known Hosts of *Legionella*

Organism	Eukaryote	Reference
Acanthamoeba polyphaga	Amoebae	Rowbotham, 1980
Acanthamoeba castellanii	Amoebae	Rowbotham, 1980
Naegleria gruberi	Amoebae	Rowbotham, 1980
Naegleria jadini	Amoebae	Rowbotham, 1980
Naegleria lovaniensis	Amoebae	Tyndall and Domingue, 1982
Acanthamoeba royreba	Amoebae	Tyndall and Domingue, 1982
Acanthamoeba palestinensis	Amoebae	Anand et al., 1983
Tetrahymena pyriformis	Ciliate	Fields et al., 1984
Naegleria fowleri	Amoebae	Newsome et al., 1985
Vahlkampfia jugosa	Amoebae	Rowbotham, 1986
Echinamoeba exudans	Amoebae	Fields et al., 1989
Hartmanella cantabrigiensis	Amoebae	Fields et al., 1989
Vermamoeba [Hartmanella] vermiformis	Amoebae	Fields et al., 1989
Hartmanella sp.	Amoebae	Fields et al., 1989
Acanthamoeba culbertsoni	Amoebae	Fields et al., 1989
Tetrahymena thermophile	Ciliate	Kikuhara et al., 1994
Dictyostelium disocideum	Amoebae	Hägele et al., 2000
Balamuthia mandrillaris	Amoebae	Shadrach et al., 2005
Caenorhabditis elegans	Nematode	Rasch et al., 2016
Willaertia magna	Amoebae	Dey et al., 2009
Diphylleia rotans[a]	Flagellate	Valster et al., 2010
Echinamoeba thermarum[a]	Amoebae	Valster et al., 2010
Neoparamoeba spp.[a]	Amoebae	Valster et al., 2010
Acanthamoeba griffini, Acanthamoeba jacobsi, Naegleria australiensis, Naegleria philippinensis, Naegleria italica	Amoebae	Hsu et al., 2011
Stylonychia bifaria[a]	Ciliate	Rasch et al., 2016
Stylonychia mytilus[a]	Ciliate	Rasch et al., 2016
Ciliophrya spp.[a]	Ciliate	Rasch et al., 2016

[a] Candidate host; in vitro studies are needed to confirm their role as environmental host.

Not all protozoa will serve as hosts under all conditions or for all strains and species of *Legionella* (e.g., Wadowsky et al., 1991), perhaps due to novel non-coding RNA expressed during predator–prey interactions (Weissenmayer et al., 2011). For example, some protozoa may be resistant to infection with *L. pneumophila* because they digest *L. pneumophila* (Amaro et al., 2015); these eukaryotes could potentially serve as biological control agents (Maita et al., 2018, as suggested by Wang et al., 2013). Some protozoa have a preference for other bacterial prey (Shaheen et al., 2019). Finally, some species of amoeba contain symbionts that do not allow *Legionella* to replicate within the host (Okubo et al., 2018; Maita et al., 2018).

The protozoan hosts for *L. pneumophila* graze on microorganisms present in the biofilm or sediments. By phagocytosis, host cells engulf and internalized prey microbes within cell membranes known as phagosomes (Abu Kwaik et al., 1998). Normally, the phagocytosed prey is delivered to lysosomes where it is digested by the acidic pH and lysosomal enzymes. Nutrients liberated through this process fuel the protozoan cell. However, many bacterial species, including pathogenic *Legionella* spp., have evolved strategies to escape digestion by the protozoan cell and persist in vacuoles within the protozoan host (Vandenesch et al., 1990). Within these vacuoles, these bacteria may multiply, especially at temperatures greater than 30°C (Buse et al., 2017; Caicido et al., 2018). Bacterial proliferation eventually kills or lyses the protozoan cell, releasing the intracellular progeny into the aquatic or soil environment (Kuiper et al., 2004). This strategy likely enables fastidious bacteria such as *L. pneumophila* to persist and compete with other bacteria in otherwise low-nutrient (oligotrophic) and inhospitable environments (King et al., 1988). As a consequence of adaptation to resist amoeba digestion, various bacteria either grow pathogenically, become benign, or form a stable symbiotic relationship with protozoa (Schmitz-Esser et al., 2010; Shu et al., 2018) as discussed further below.

Effects of the Protozoan Host on Virulence and Stress

Over millennia, free-living protozoa have contributed to the development of a repertoire of virulence and stress-response genes in *Legionella* and other amoeba-resisting bacteria (Guimaraes et al., 2015; Koubar et al., 2011; Trigui et al., 2015). Considerable horizontal gene transfer between amoeba and their internalized bacteria (Guimaraes et al., 2015) also contributes to ongoing changes in traits within legionellae. The host and the conditions for culturing legionellae may also affect its infectivity, such as if cells are VBNC-like, in the replicative or the transmissive stage of *Legionella*'s life-cycle (Fonseca and Swanson, 2014), or as short rods versus filamentous morphologies (Garduño et al., 2002; Vandenesch et al., 1990). Adaptation of *L. pneumophila*'s lipid A cell surface is one of a series of factors that affects its ability to infect host amoebae (Albers et al., 2007). As discussed previously, not describing and controlling the cell form(s) used in infection studies could well be contributing to the differing views reported in the literature.

The increase in infectivity following *L. pneumophila* growth in amoebae may depend on the protozoan host and the animal model used, although the mechanism of this increased virulence is unknown. While no difference was seen in the infectivity of guinea pigs via aerosols of *L. pneumophila* cells grown in co-culture with an *Acanthamoeba* spp. compared to pure culture cells from agar plates (Vandenesch et al., 1990), *Acanthamoeba castellanii*- and *Vermamoeba vermiformis*-associated *L. pneumophila* were described as more pathogenic in macrophages and in a mouse model than an equal number of non-amoeba-associated *L. pneumophila* (Brieland et al., 1997; Cirillo et al., 1994). For example, *L. pneumophila* grown in *Acanthamoeba* are some 100-fold more infectious in epithelial cells and ten times more infectious in macrophages or other cell lines than agar-grown *L. pneumophila* (Cirillo et al., 1994, Garduño et al., 2002). Importantly, after intracellular replication in free-living protozoa, higher resistance has been document-

ed for *L. pneumophila* stressed by heat, oxidants, acids, osmotic shock (Kwaik et al., 1997), biocides (Barker et al., 1992, Berk et al., 1998), and antibiotics (Barker et al., 1995, Garduño et al., 2002). Other cellular differences between co-cultured *L. pneumophila* and *L. pneumophila* grown alone on agar include differences in cellular fatty acid composition (Barker et al., 1993; Vandenesch et al., 1990). However, a limitation of each of these comparative studies is that it is unclear whether the agar-grown cell samples contained exponential-phase "replicative" bacteria that are readily degraded in lysosomes, post-exponential-phase "transmissive" bacteria that evade lysosomes, or a mixture of the two.

Role of Temperature

Legionellae have been observed in environments ranging from 0°C to 45°C, indicating that legionellae are psychrophilic to mesophilic (Wullings and van der Kooij, 2006). However, most of the described species of the genus *Legionella* are mesophilic and grow between 25°C and 43°C under laboratory conditions (Garrity et al., 2005). Under environmental conditions, growth of *L. pneumophila* has been observed between 25°C and 45°C (Buse et al., 2017; Tison et al., 1980; van der Kooij et al., 2016; Wadowsky et al., 1985; Yee and Wadowsky, 1982). Since the growth of *L. pneumophila* in these environments largely depends on amoebae, studies have also focused on *L. pneumophila* growth in protozoan hosts at different temperatures. *L. pneumophila* proliferate in *Acanthamoeba palestinensis* and *A. castellanni* at 25°C and 35°C, respectively, but are digested by the amoeba at 15°C and 20°C (Anand et al., 1983; Ohno et al., 2008), which is consistent with generally low levels of *Legionella* observed in natural environments at these lower temperatures.

The optimal temperature range for *L. pneumophila* to express several factors that promote infection and transmission is between 25°C and 30°C, not 37°C or higher. These factors include flagellar-based motility (Ott et al., 1991); PilD, a critical component of Type II secretion and Type IV pili, two machines that equip *L. pneumophila* to move across surfaces by sliding motility (Stewart et al., 2009); and LvhB2, a virulence factor exported by Type II secretion that enhances *L. pneumophila* infection of host cells at 25°C, but not 37°C (Ridenour et al., 2003). A functional Type II secretion system also increases *L. pneumophila* survival in water at temperatures of 17°C and below and bacterial replication in amoebae at 22°C to 25°C (Söderberg et al., 2004).

Studies differ in the maximum temperature observed for *L. pneumophila* growth. Some researchers observed growth up to 45°C (Kusnetsov et al., 1996; Tison et al., 1980), whereas others did not observe growth at 42°C or higher (Ohno et al., 2003; van der Kooij et al., 2016). Van der Kooij and colleagues (2016) observed that *L. pneumophila* serogroup 1 strains of different sequence types had different optimum and maximum growth temperatures when grown under drinking water conditions in a biofilm monitor. In addition, using the same biofilm monitor, *L. pneumophila* was not capable of growth in the naturally formed biofilm with temperatures greater than 41°C. A 2-log lower *V. vermiformis* host count at 42°C compared to 38°C indicated that the absence of a thermotolerant host at 42°C prevents proliferation of *L. pneumophila* in this system (van der Kooij et al., 2016)2016. Indeed, different *L. pneumophila* strains have different optimal temperatures when grown in protozoan hosts (Buse and Ashbolt, 2011), and the protozoan community composition also varies with temperature in drinking water systems (Valster, 2011). Thus, both the strain of *L. pneumophila* and host protozoan diversity affect the temperature range for growth of *L. pneumophila*.

At temperatures greater than 50°C, the number of cultivable *L. pneumophila* declines. The time required to reduce the concentration of viable bacteria by 90 percent is referred to as the decimal reduction time. Decimal reduction times between 100 and 1,000 minutes were observed for *Legionella* at

50°C (van der Kooij, 2014). The lowest reduction time (100 minutes) was observed for *L. pneumophila* precultured with natural microbiota. Moreover, these decimal reduction times decreased to around two minutes at 60°C for *L. pneumophila* pure cultures grown under natural conditions (Dennis et al., 1984; Schulze-Robbecke et al., 1987; van der Kooij, 2014). No cultivable *L. pneumophila* were observed after heat shock treatment for 10 minutes at 70°C with pure cultures of different *L. pneumophila* strains (Allegra et al., 2008). In contrast, metrics of viability (membrane-intact cells and adenosine triphosphate or ATP present) could still be measured for most strains, and, when the suspension was subsequently incubated with a protozoan host, *L. pneumophila* was cultivated. Membrane integrity can be a poor indicator for viability in *L. pneumophila* (Hammes et al., 2011, Wullings et al., 2016), and free ATP can still be present after cell death (Nescerecka et al., 2016). Nevertheless, the capacity to culture bacteria from a protozoan host demonstrates that at least some *L. pneumophila* cells remained viable after heat-shock treatment for 10 minutes. Unfortunately, a decimal reduction time was not calculated. An earlier study of several *L. pneumophila* strains showed that the decimal reduction time at 70°C varied between 1.1 and 2.6 minutes (Stout et al., 1986), meaning that 10 minutes of exposure to 70°C would be expected to result in a 3.8- to 9.1-log reduction. Therefore, the Allegra et al. (2008) report that *L. pneumophila* cells survived 10 minutes of treatment at 70°C is consistent with previous observations.

Inactivation of pure cultures of ten different *Legionella* species, eight different *L. pneumophila* serogroups and one to five different *L. pneumophila* strains showed that the decimal reduction times at 60°C ranged from 2 to 5 minutes (Stout et al., 1986). This difference in decimal reduction time was observed between species, serogroups, and strains, but was only determined by loss of culturability, not by other measures of viability loss (hence, some cells may be VBNC-like). In addition, Allegra and colleagues (2008) also observed that the different *L. pneumophila* strains showed different reduction curves for the membrane-intact cells when treated at 70°C (see Figure 2-8). Thus, the reduction times after temperature disinfection can be strain-, serogroup-, and species-dependent. Interestingly, Allegra et al. (2011) showed that repeated thermal shocks actually selected for heat-resistant *L. pneumophila* strains.

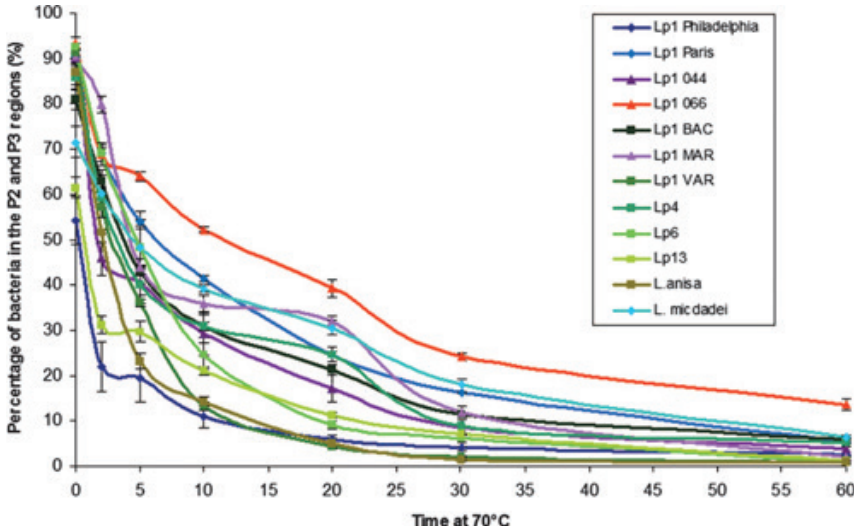

FIGURE 2-8 Inactivation curves for *L. anisa* and *L. micdadei* strains, different *L. pneumophila* (Lp) serogroups (1, 4, 6 or 13), and different strains of *L. pneumophila* serogroup 1 (Philadelphia, Paris, 044, 066, BAC, MAR and VAR) after exposure to 70°C. For each strain, the result at each time point shown is the percentage of bacteria detected in both the P2 (viable, culturable cells) and P3 (VBNC-like cells) regions and is expressed as the mean ± standard error from two to five independent experiments. SOURCE: Allegra et al. (2008).

Most recently, Cervero-Aragó and colleagues (2019) investigated two *L. pneumophila* strains for more than 80 days using a combination of cell-based viability indicators with cells incubated at 55°C, 60°C, and 70°C. Culturability was lost after 3 to 8 hours, 60 minutes, and less than 2 minutes, respectively; whereas, based on viability indicators, a 4-log reduction was achieved only after 150, 8 to 15, and 1 to 4 days, respectively. To investigate cells in a VBNC-like state, Cervero-Aragó et al. (2019) evaluated the infectivity of these heat-shocked *L. pneumophila* in amoebae and a lung macrophage cell line (THP-1). Infectivity lasted for at least 85 days at 55°C and 60°C and for up to 8 days at 70°C, albeit with reduced efficiency. Cervero-Aragó et al. (2019) concluded that a prolonged thermal regime at or above 60°C at the central parts of warm-water building systems is not only effective against culturable *L. pneumophila* but also against VBNC-like cells.

Since *L. pneumophila* seems to multiply mainly in protozoan hosts, the effect of high temperatures on possible protozoan hosts for *L. pneumophila* might also contribute to eradication of *L. pneumophila*. Most protozoan host species of the genus *Acanthamoeba* cannot multiply at temperatures above 37°C (de Jonckheere, 1980). However, another protozoan host for *L. pneumophila* (such as *V. vermiformis*) continues to multiply above 50°C (Kuchta et al., 1993; Rohr et al., 1998). Cervero-Arago et al. (2013) showed that trophozoites of two *Acanthamoeba* strains and two *V. vermiformis* strains, isolated from drinking water, had a decimal reduction time of 7.2 to 11 minutes at 50°C and less than 0.34 to 1.4 minutes at 60°C. In contrast, the decimal reduction time of cysts of these protozoan species varied between 30 and 76 minutes at 50°C and between 4.7 and 10.7 minutes at 60°C, with *Acanthamoeba* strains being the most thermotolerant. These reduction times at 60°C are considerably longer than for *L. pneumophila*, implying that decimal reduction times for *L. pneumophila* inside cysts are likely to be longer than for planktonic *L. pneumophila* cells. Indeed, Storey et al. (2004b) showed persistence of *L. pneumophila* within cysts after 10 minutes at 80°C.

Role of Nutrients

Growth of legionellae in defined media demonstrate that these bacteria require specific nutrients, including certain amino acids and ferric iron (George et al., 1980; Ristroph et al., 1981). Indeed, legionellae are readily recovered in relatively nutrient-rich reclaimed wastewaters (as reviewed by Caicedo et al., 2019). Conversely, free cells of *L. pneumophila* cannot compete successfully with oligotrophic bacteria in nutrient-poor aquatic environments such as drinking water (van der Kooij, 2014). As described above, *L. pneumophila* circumvents this problem by multiplying in protozoan hosts, which provide its required nutrients. Since these protozoan hosts graze on bacteria, the concentration of protozoa depends on the concentration of prey bacteria that are present. A clear correlation between biofilm concentration and numbers of cultivable *L. pneumophila* has been observed in a biofilm monitor fed with unchlorinated drinking water types that differ in assimilable organic carbon (AOC) concentration and inoculated with *L. pneumophila* (Learbuch et al., 2019; van der Kooij et al., 2017). The biofilm concentration also correlates with the AOC concentration in the drinking water (van der Kooij et al., 2017). This implies that the *Legionella* numbers in drinking water are indirectly correlated to the AOC concentration of the water. Based on these data, the authors deduced for drinking waters sourced via riverbank infiltration a threshold biofilm concentration of 50 μg ATP/cm^2 and a threshold AOC concentration of 1 μg C/L to initiate growth of *L. pneumophila* in the product drinking water.

Notably, an AOC concentration of less than 1 μg C/L in drinking water is extremely low and cannot be achieved by drinking water plants in the United States (Volk and LeChevallier, 2000). Even in the Netherlands, where microbial growth in drinking water systems is limited by controlling AOC

concentrations instead of using a disinfectant residual, the majority of treatment plants produce drinking water with AOC concentrations above 1 µg C/L (Paul van der Wielen, personal communication). Others have not observed a relationship between organic carbon and *L. pneumophila* numbers in drinking water supplemented with different fulvic acid concentrations (Williams et al., 2015), indicating that other environmental factors (e.g., host protozoan concentration) contribute to *L. pneumophila* growth as well.

A relationship between several minerals (e.g., iron, zinc, calcium, manganese, magnesium) and *Legionella* numbers in premise plumbing systems has been observed (van der Kooij, 2014, and references therein), although findings are not consistent among studies. For instance, Vickers and colleagues (1987) observed that calcium and magnesium, but not iron and zinc, correlated with *L. pneumophila* in hospital water systems, whereas Bargellini and colleagues (2011) observed that iron and zinc correlated with *L. pneumophila* in hot-water systems of hotels and private homes. The role of ferric iron in growth of *L. pneumophila* has been studied intensively; it is a prerequisite for growth of *L. pneumophila* in chemically defined media (Reeves et al., 1981; States et al., 1985; Warren and Miller, 1979). The addition of the iron chelator lactoferrin reduces viability of *L. pneumophila* (Goldoni et al., 2000). In addition, ferric/ferrous ions also promote *L. pneumophila* virulence (Allard et al., 2009). Iron overload has also been associated with increasing *Legionella* infectivity rates of amoebae and macrophages as indicators of increased virulence (Buracco et al., 2017; James et al., 1999). On the other hand, excessive iron inhibits *Legionella* biofilm formation (Hindré et al., 2008). The effect of other mineral elements on the growth of *L. pneumophila* in premise plumbing is still not well understood (Borella et al., 2005).

Low nutrient availability in artificial media inhibits *L. pneumophila* growth, generating VBNC-like cells. As discussed previously, whether *Legionella* forms VBNC cells is controversial. VBNC-like *L. pneumophila* cells persisting in drinking water for more than 100 days that can be resuscitated by amoeba co-culture have been described (Steinert et al., 1997). It remains difficult, however, to make definitive conclusions because the lack of growth in the absence of a protozoan host was not proven (only that *L. pneumophila* was below a certain detection limit). Still, others have made similar observations in which resuscitation through a protozoan host recovered the culturability of *L. pneumophila* on agar media (Alleron et al., 2008; Ducret et al., 2014; Garcia et al., 2007; Hwang et al., 2006). Furthermore, it was observed that detection of *L. pneumophila* from environmental samples is improved when a co-culture method with a protozoan host is used (Garcia et al., 2007; La Scola et al., 2001; Sanden et al., 1992; Schalk et al., 2012). This is strong evidence that not all viable *L. pneumophila* strains in the environment will be cultivated when using artificial media.

Legionella in the Natural Environment

Aquatic Environment

Soon after *L. pneumophila* was first isolated and described, the organism was observed at low numbers (approximately 1 percent of total bacterial community) in water from different lakes and rivers in the United States using DFA techniques and isolated by infecting guinea pigs (Fliermans et al., 1979, 1981). The frequency of *L. pneumophila* isolation correlated with water temperature, and more strains could be isolated from surface waters in summer (Fliermans et al., 1979). Around the world, legionellae were identified among the natural microbiota in surface waters (e.g., Carvalho et al., 2007, 2008; Ortiz-Roque and Hazen, 1987; Parthuisot et al., 2010) or groundwater (Costa et al., 2005; Riffard et al., 2001). These studies show in general that legionellae numbers are low (less than 10^3 CFU/L). Detected only sporadically, *L. pneumophila* is not among the dominant *Legionella* species in surface waters and groundwater that have

water temperatures below 25°C. In contrast, *L. pneumophila* predominated among the legionellae isolated from natural hydrothermal vents and hot springs where water temperatures were above 27°C, reaching numbers up to 10^6 CFU/L (Marrao et al., 1993; Verissimo et al., 1991). In an acidic hot spring with a temperature of 30°C to 47°C and a pH of 2.7, 16S rRNA gene sequences of *L. pneumophila* were not detected, indicating that the low pH selected for other *Legionella* species (Sheehan et al., 2005). *L. pneumophila* has been identified in natural water from thermal springs used in spas, a suspected source for Legionnaires' disease (van Heijnsbergen et al., 2015). It should be noted that the protozoan hosts for *L. pneumophila* also reside in natural aquatic environments (reviewed in Plutzer and Karanis, 2016).

Soil Environment

Legionellae also inhabit soil. For example, a large number of legionellae isolates (n=114) belonging to 12 different *Legionella* species were obtained from soil samples in Thailand (Travis et al., 2012). Moreover, *L. longbeachae*, another causative agent of Legionnaires' disease, is mostly isolated from potting mixes and gardening soil (Whiley and Bentham, 2011 and references therein), although it resides in aquatic environments as well (Joly et al., 1984; Marrie et al., 1994). In contrast to *L. pneumophila*, *L. longbeachae* contains genes that encode 12 cellulolytic enzymes, which together may fully degrade cellulose. Accordingly, *L. longbeachae* is equipped to metabolically degrade plant material (Cazalet et al., 2010), supporting the notion of its natural habitat being associated with degrading plant matter.

Morris and colleagues (1979) isolated *L. pneumophila* from mud and sand sediments sampled from the bottom of a stream impacted by thermally polluted water. Furthermore, *L. pneumophila* was detected in these soil samples by fluorescent antibody staining, although the microbes could not be cultivated on agar media or after guinea pig inoculation. Others have detected *L. pneumophila* in soil, in one case throughout the year in the same garden soil (Travis et al., 2012; van Heijnsbergen et al., 2016). In Australia, the same unusual *L. pneumophila* strain type was isolated from a Legionnaires' disease patient and from soil in the patient's work area, implying a direct link between soil and disease (Wallis and Robinson, 2005). Likewise, natural soil has been identified as a possible source for various cases of Legionnaires' disease (van Heijnsbergen et al., 2015). Although different *Legionella* species have been isolated from soil, and soil or dust contamination of engineered systems has been associated with outbreaks (van Heijnsbergen et al., 2016), the ecology of legionellae in soil has not been studied intensively. Free-living protozoa known to be hosts for *L. pneumophila* (e.g., *V. vermiformis* and *Acanthamoeba* spp.) are also common in soil samples (Denet et al., 2017; Tyml et al., 2016). Hence, *L. pneumophila* in the soil environment likely replicate within protozoan hosts, although proof for this phenomenon is still lacking.

Legionella in Engineered Environments

The growth of pathogenic legionellae to problematically high densities (Hamilton et al., 2019) seems to be favored in various engineered environments that support free-living protozoa associated with biofilms and that generate aerosols (Buse et al., 2012). The engineered environments that support *Legionella* growth include drinking water treatment plants, plumbing within buildings (i.e., premise plumbing), cooling towers, wastewater treatment plants that receive warm industrial effluents (Loenenbach et al., 2018), and a myriad of devices that operate with warm water including hot tubs. As shown in Figure 2-9, these compartments are a potential source of Legionnaires' disease if contaminated aerosols are generated that can be inhaled or aspirated by people in their vicinity.

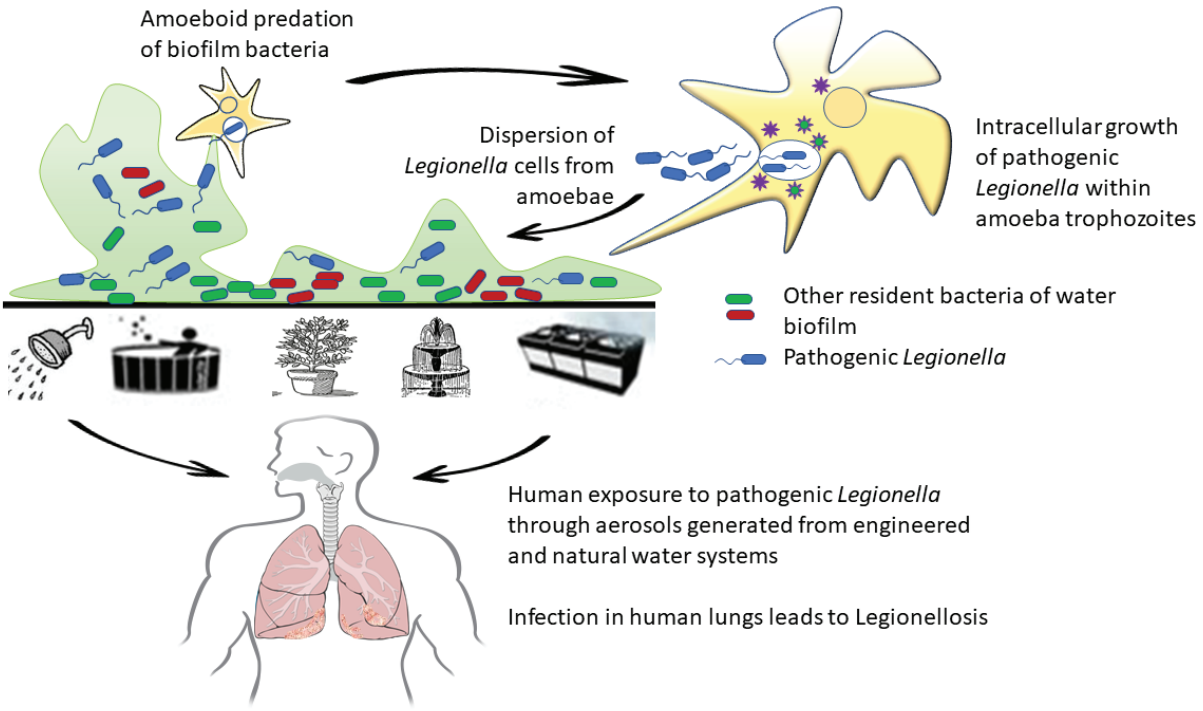

FIGURE 2-9 Major pathways leading to *Legionella* infections in engineered environments.
SOURCE: Courtesy of Mohamed Shaheen.

Drinking Water Distribution Systems and Premise Plumbing

Various species of legionellae have been detected in drinking water (Buse et al., 2012; Donohue et al., 2014; Hull et al., 2017). *Legionella* can enter drinking water systems by many possible routes, starting with source water that is contaminated by industrial, thermally elevated wastewater. For example, *Legionella* can grow within free-living protozoa associated with sand filters used in drinking water treatment and within amoebae that pass through drinking water treatment into distribution (Loret and Greub, 2010; Lu et al., 2016). Lu et al. (2015) provided evidence for atmospheric (soil/dust) ingress of *Legionella* through air vents into drinking water reservoirs and growth within reservoir sediments. *Legionella* may also enter drinking water distribution systems during construction (Francois Watkins et al., 2016; Knox et al., 2016; hypothesized in Mermel et al., 1995; Stout et al., 2000) or main breaks (as suggested in Rhoads et al., 2017a).

Drinking water distribution systems containing free-living protozoa harboring pathogenic legionellae can also "seed" premise plumbing; subsequent growth in dead-ends and other stagnant regions may lead to sporadic cases and outbreaks of Legionnaires' disease (Beer et al., 2015; Hamilton and Haas, 2016; Schoen and Ashbolt, 2011). In particular, the last few distal meters of piped water in premise plumbing are likely zones where problematic concentrations of *L. pneumophila* develop (i.e., 10^5 to 10^6 CFU/L; Hamilton et al., 2019; Schoen and Ashbolt, 2011). *L. pneumophila* has been isolated from hot/cold mixing valves, tap aerators, plastic shower hosing, and shower heads (Collins et al., 2017; Proctor et al., 2018); household "cold"-water storage tanks (Peter and Routledge, 2018); and stagnant hot-water lines within an optimal temperature window for intracellular growth (i.e., 28°C to 45°C) (Proctor et al., 2017). The many reasons for this proliferation are discussed below.

Stagnation. Stagnant areas in premise plumbing support more cultivable legionellae (including *L. pneumophila*) than other parts of premise plumbing (Fisher-Hoch et al., 1982; Tobin et al., 1981). Ciesielski et al. (1984) showed that hot-water tanks with stagnant water support higher *L. pneumophila* numbers (10^5 to 10^6 CFU/L) compared to hot-water tanks in which water was continuously replaced (less than 10^4 CFU/L). Compared to non-stagnant water, stagnant water has lower or no disinfectant residual (Fisher-Hoch et al., 1982; Wang et al., 2012), lower water temperatures (Patterson et al., 1994), higher concentrations of organics (LeChevallier et al., 1996; Wang et al., 2012), lower dissolved oxygen concentrations (Wang et al., 2012), higher biomass concentrations (Lautenschlager et al., 2010), altered microbial community composition (Dai et al., 2018a; Lautenschlager et al., 2010), and higher numbers of protozoan hosts (Wang et al., 2015b)—factors that all influence *L. pneumophila* growth.

Corrosion. The impact of corrosion products on *Legionella* proliferation is multifaceted (Brazeau and Edwards, 2013). By consuming residual disinfectant, these compounds create a more favourable environment for *Legionella* growth. Increased bioavailability of various metal corrosion products, such as iron, may also upregulate virulence in legionellae, stimulate general biofilm growth (Buse et al., 2012), and contribute to *Legionella* growth in hot-water heaters (Dai et al., 2018b; Ji et al., 2017; Proctor et al., 2017). Iron released during the recent massive corrosion event in Flint, Michigan, contributed to loss of chlorine residual and, as a required nutrient (Reeves et al., 1981; States et al., 1985; Warren and Miller, 1979), this metal was also hypothesized to stimulate *Legionella* growth (Rhoads et al., 2017a). Van der Lugt et al. (2017) also recently reported that iron rust in stainless-steel shower heads resulted in increased *Legionella anisa* plate counts. Corrosion products can also promote heterotrophic biofilm growth by producing electron donors, such as hydrogen, and by stimulating autotrophic metabolism and fixation of organic carbon in the system (Rhoads et al., 2017b).

Pipe Materials. Pipe material may influence growth of *L. pneumophila*. For example, rubber material in a model pipe system enhanced growth of *L. pneumophila*, except when a biocide (thiuram) was present in the rubber material (Niedeveld et al., 1986). Plastic pipe materials can also enhance growth of *L. pneumophila*, especially those used in premise plumbing, such as soft PVC (PVC-P), polyethylene, polypropylene, or polybutylene materials (Rogers et al., 1994a,b; van der Kooij et al., 2002). The biofilm concentration on each type of pipe material correlates with *L. pneumophila* load (Learbuch et al., 2019). Therefore, pipe materials most likely affect *L. pneumophila* growth indirectly: higher biofilm concentrations support more protozoan hosts, which generate higher counts of *L. pneumophila* (van der Kooij et al., 2017). European standardized laboratory tests have demonstrated that, compared to an inert material such as glass, rubber (natural and synthetic), soft PVC (i.e., PVC-P), polyethylene, polypropylene, and polybutylene significantly enhance microbial growth (Hambsch et al., 2014) because of the growth-promoting organic compounds that these materials release. In contrast, stainless steel, PVC-C, and PVC-U did not enhance growth of *L. pneumophila* in these laboratory tests. A field study of several buildings demonstrated that the highest cultivable legionellae numbers were present in the biofilm on rubber components of taps (van Hoof et al., 2014), consistent with various laboratory test results.

In premise plumbing, the impact of copper pipes on legionellae is not consistent among studies, possibly due to differences in biofilm microbiota and the physiological status of cells. Several laboratory studies report that copper inhibits growth of *L. pneumophila* (e.g., Learbuch et al., 2019; Rogers et al., 1994b; Schoenen et al., 1988). In addition, Danish buildings with copper premise plumbing showed lower cultivable legionellae counts than buildings with iron pipes (Pringler et al., 2002). In contrast, others observed enhanced growth of *L. pneumophila* on copper compared to PVC-U or PVC-C (Buse et al., 2014a,b; Gião et al., 2015; van der Kooij et al., 2002). Likewise, by comparing bacteria growing in

tubing downstream of biofilm reactors with copper versus PVC-U coupons, Lu et al. (2014) also noted that injected *L. pneumophila* actually survive better downstream of copper. A companion paper by the same group (Buse et al., 2014a) further indicated that the copper coupons were colonized by and released a greater number of *L. pneumophila* when co-inoculated with *Acanthamoebae polyphaga* and measured by qPCR, but *L. pneumophila* were only cultivable from PVC-U coupons.

There are several possible explanations for the apparent enigma of net effects of copper plumbing on *Legionella*. First, van der Kooij and colleagues (2005) observed that new unused copper material initially inhibited growth of *L. pneumophila* due to the release of copper ions, but when the copper material was corroded, release of copper ions was reduced and inhibition of *L. pneumophila* no longer occurred. Interactions of the copper pipe with the local water chemistry is also important to consider. Proctor et al. (2017) noted that benefits of copper pipe for depressing *L. pneumophila* levels were only apparent at or below 41°C. Above 53°C, *L. pneumophila* were no longer detectable, and thus pipe material did not matter. Buse et al. (2017) noted that a higher pH (greater than 8.2), which limits dissolution, can also limit antimicrobial activity of copper pipe. Build-up of corrosion byproducts over time also limits the antimicrobial activity of copper toward *Legionella* (van der Kooij et al., 2005). In addition to having stronger antimicrobial properties than solid Cu, free Cu^{2+} in solution can induce other reactions, such as corrosion and associated hydrogen production, which could indirectly impact *Legionella* (Proctor et al., 2017; Rhoads et al., 2017b).

Copper might also induce a VBNC-like state for *L. pneumophila*, as has been suggested for *Pseudomonas aeruginosa* (Flemming et al., 2014). Induction of a VBNC-like state through copper exposure decreased the number of *L. pneumophila* detected by cultivation (Learbuch et al., 2019; Rogers et al., 1994a; Schoenen et al., 1988; van der Kooij et al., 2002), but not the number quantified by DNA-based methods (Buse et al., 2014a,b; Gião et al., 2015). Consistent with this hypothesis, after batch incubations with copper ions, Proctor et al. (2017) reported sharper decreases in *L. pneumophila* numbers by plate counts versus qPCR. Also, copper (and other) materials influence the microbial composition of premise plumbing biofilms (Buse et al., 2014a; Proctor et al, 2018), with copper resulting in less biofilm growth than various hard and soft plastics (Proctor et al., 2018; van der Kooij et al., 2017). Interestingly, while less biofilm may accumulate on copper materials than on plastics, the types of bacteria and amoeba present could be more supportive of *L. pneumophila* growth than those on plastics (Buse et al., 2014a,b; Gião et al., 2015). In particular, *V. vermiformis* is the *L. pneumophila* host most often associated with warm- and hot-water (largely copper-pipe) systems (Buse et al., 2017; Ji et al., 2017). Hence, along with biofilm concentration, the species composition of the biofilm is important for growth of amoebae that favor *L. pneumophila* replication. Overall, *L. pneumophila* growth appears enhanced in biofilms dominated with α-Proteobacteria, key prey for protozoan hosts (van der Kooij et al., 2018).

Once within the complex plumbing of a large building, *L. pneumophila* may persist given the right combinations of temperature, stagnation, and subsequent loss of residual disinfectant, often exacerbated by the presence of iron oxides/hydroxioxides/humics (Butterfield et al., 2002) and other pipe corrosion products (Rhoads et al., 2017b). *L. pneumophila* strains have remained detectable in simulated building water systems for a long time (i.e., up to 2.4 years) (Paszko-Kolva et al., 1992; Skaliy and McEachern, 1979; Wadowsky and Yee, 1985), with the one apparent clone in buildings causing outbreaks over decades (Garcia-Nunez et al., 2008). This prolonged survival in water has been attributed to the organism's ability to produce and store poly-3-hydrobutyrate, a carbon/energy source when nutrients are scarce (James et al., 1999; Mauchline et al., 1992). Recently, Shaheen and Ashbolt (2019) showed that viable cells of a *L. pneumophila* serogroup 1 strain remained in a dormant-like state associated with amoebae for over two years in drinking water. Such persistence may be associated with the expression of a Type II

secretion system, a transport mechanism that promotes bacterial growth at ambient drinking water temperatures (less than 25 °C) (Söderberg et al., 2004). In cold water, *Legionella* persist within trophozoites and acquire nutrients sufficient for maintenance from the host, but the population does not expand. Cold-water systems are unlikely to result in cases of legionellosis, except in warm climates where the water temperature exceeds 25°C for extended periods.

Cooling Towers and Industry Wastewater Treatment Works

Cooling towers provide a favorable environment for proliferation of *Legionella* due to not only their warm water temperatures but also the large surface area available for biofilm colonization. In addition, cooling towers can broadly disperse aerosols, from hundreds of meters to kilometres away (Nguyen et al., 2006). Cooling towers operate within the temperature window that supports *L. pneumophila* growth within amoebae (Berk et al., 2006; Canals et al., 2015; Critchley and Bentham, 2007; Hammer, 2018; Llewellyn et al., 2017; Nguyen et al., 2006; Scheikl et al., 2016), making control reliant primarily upon the use of biocides. It is also critical to position building air intakes well away from cooling tower-generated aerosols, while accounting for thermal inversion and other atmospheric phenomena (Engelhart et al., 2008).

Extensive outbreaks of legionellosis have also tracked to activated sludge treatment of pulp and paper mill and brewery effluents, which are typically not disinfected and are within 30°C to 40°C (Maisa et al., 2015; Nygård et al., 2008). On the other hand, conventional municipal wastewater treated by the activated sludge process typically has low levels of legionellae, probably in part due to the temperatures being below 30°C (Caicedo et al., 2018).

Hot Tubs

Public and private hot tubs are typically maintained at 38°C to 44°C, a favorable temperature range for *L. pneumophila*. A common feature linked to *Legionella* growth in hot tubs is biofilm build-up on internal plumbing surfaces that periodically contain stagnant warm water for extended periods (hours to days) (Costa et al., 2010). Sudden mobilization with entrained air and/or water circulation can aerosolize *Legionella* cells (likely associated with biofilm and amoebae) and lead to human infections (Fallon and Rowbotham, 1990; Leoni et al., 2018). Because hot tubs are inherently warm, there is a heavy reliance on biocides to suppress microbial growth (e.g., Qin et al., 2013). However, biocides can by no means eradicate all resident microbes, and these chemicals can also shape the microbial communities inhabiting the hot tubs. Hot tubs are *Legionella* infection risks not only for their users but also for attendants and patrons who pass down-wind of aerosols (Costa et al., 2010; Hamilton et al., 2018).

Other Devices

As with any device exposed to air and moisture, humidifiers and nebulizers can support biofilm growth and associated legionellae and deliver aerosols to susceptible individuals, particularly in healthcare settings (Kyritsi et al., 2018; Mastro et al., 1991; Yiallouros et al., 2013). For example, supermarket vegetable misters have been sources of Legionnaires' disease (Barrabeig et al., 2010). Other devices that generate aerosols from stored/stagnating warm water sources and have been associated with cases of

Legionnaires' disease include car windshield wiper fluids and sprayers (Wallensten et al., 2010), car wash facilities (Baldovin et al., 2018), and decorative fountains (Decker and Palmore, 2013).

Plant Growth Media and Bagged Compost

The major non-water medium associated with cases of Legionnaires' disease is garden soil and compost materials, which most often promote the growth and detection of *L. longbeachae* (Currie et al., 2014; Whiley and Bentham, 2011). However, *L. longbeachae* is probably under-reported even more than non-serogroup 1 *L. pneumophila* and may also occur within a range of environments discussed above such as cooling towers (Bacigalupe et al., 2017; Thornley et al., 2017).

EXPOSURE PATHWAYS

As pathogenic legionellae grow to high concentrations in free-living protozoa associated with biofilms, infectious *Legionella* cells may be released in aerosols in various forms: as free cells, cells within biofilm fragments, or cells associated with free-living protozoan trophozoites, cysts, or expelled vesicles (membrane-bound structures containing undigested materials) (see Figure 2-7; Lau and Ashbolt, 2009; Shaheen and Ashbolt, 2017). While *Legionella* infections are the most frequently identified etiologic agents from environmental aerosol exposures, other amoeba-resisting bacterial pathogens may also cause infections but go undetected because of inadequate methodologies used to identify agents (Lamonth and Greub, 2010; Lienard et al., 2017).

Common exposure pathways include breathing in aerosols in the 2- to 10-μm size range generated by showers, hot tubs, humidifiers, spray misters, cooling towers, car washers, windshield wiper spray, aeration basins used in wastewater treatment, and water (decorative) features and fountains. Aspiration of drinking water is another exposure pathway. A particular feature of *L. longbeachae* infections is the association with aerosols generated from wood and bark residuals and poorly matured compost sold as potting media in sealed plastic bags (Steel et al., 1990), since *L. longbeachae* and its supporting free-living protozoa hosts thrive in the biofilm environment associated with warm, partly decaying wood residuals.

Most reports of Legionnaires' disease from building exposures involve hot water mixed with cold water used for showering or held in hot tubs. Cold tap water that has warmed, such as in humidifiers, or aspirated stagnant drinking water can also be problematic. Features that increase the likelihood of significant exposures include the use of hot- or cold-water storage tanks in buildings, aerators on taps, water conserving (finer misting) showerheads, and high-rate aeration of hot tubs.

Notably, most cysts of free-living protozoa are too large to reach the lower respiratory tract (i.e., they are greater than 10 μm). Larger biofilm fragments are also less likely to reach the sites for lung macrophage infection. In contrast, amoebae are known to expel cells and food in membrane-bound vesicles, for which a few hundred infectious *L. pneumophila* cells have been estimated per vesicle (Shaheen and Ashbolt, 2018), making *Legionella*-encapsulated vesicles a likely but rarely studied environmental form of exposure. Also, infected trophozoites may rupture within the airways, releasing several hundred free bacterial cells (Buse and Ashbolt, 2012) within the respiratory size. In support of amoeba-associated infectious *L. pneumophila* cells is the report of *Acanthamoeba* antibodies in people with legionellosis (C. Chappell, personal communication), but this association may be accidental, given the high likelihood for people to have antibodies to this genus of environmental amoebae (Chappell et al., 2001). Unfortunately, neither the biochemical nor genetic attributes that promote *L. pneumophila* survival in aerosols have been

identified, despite the large number of cases and isolates obtained from outbreaks (e.g., Bennett et al., 2014).

Physicochemical conditions of the aerosols may impact domestic, commercial, and industrial human exposures. For example, lung deposition may be more likely for bacteria in an isotonic solution (close to 0.9 percent salinity or 1,100 milliSiemens [mS]/cm^2) (Haddrell et al., 2014). This level is typical of hot-tub aerosols, which are around 1,200 mS/cm^2 compared to 4,000 mS/cm^2 in cooling towers and less than 200 mS/cm^2 in tap water. Hence, aerosols from hot tubs and perhaps cooling towers may be more likely to reach deep into the lungs (Richard Bentham, Flinders University, Adelaide, personal communication, May 14, 2018). The likelihood of aerosol entry into the lungs is further enhanced in hot tubs because people sitting in or near the units breathe in close proximity to the water surface (Moore et al., 2015). If atmospheric conditions such as relative humidity and wind direction are favorable, then *Legionella*-containing aerosols may infect people some tens of kilometers downwind (Nygård et al., 2008).

From epidemiology studies of Legionnaires' disease in the United States (reviewed by Garrison et al., 2016), the common known pathways for exposure appear to be from potable water within buildings and from cooling tower aerosols, followed by hot tubs, fountains, and other devices. Among the building categories, hotels and resorts, long-term care facilities such as nursing homes, and hospitals are the most likely to be associated with an outbreak of Legionnaires' disease. However, given that most cases of Legionnaires' disease are sporadic (see Chapter 3) and the sources of such exposures are not identified, the relative disease burden from cooling towers, from homes and larger buildings, or from other engineered water features has yet to be determined.

CONCLUSIONS AND RECOMMENDATIONS

L. pneumophila is now the leading cause of reportable drinking water-associated disease in the United States. To reduce this significant healthcare burden, a deeper understanding of *Legionella* ecology, the genetic traits that equip *Legionella* strains to colonize engineered water systems, and how *Legionella* survive in aerosols and thrive in the human lung is required. These and other important topics for future research are discussed in greater detail below.

There is a need to better understand the mechanistic pathways for the development of Pontiac fever, and what roles the pathogen, endotoxins, *Legionella*-harboring amoebae, or other exposures play in disease pathogenesis. Additional studies aimed at understanding the differences in *Legionella* species characteristics associated with Pontiac fever are also needed. Because Pontiac fever is associated with less mortality, focused studies examining this clinical entity have been limited to date. Pontiac fever reporting occurs primarily through outbreak investigations, which limits assessments of true incidence, population risk, and an understanding of the relationship between certain *Legionella* species and serotypes and disease manifestations. There is a need to develop improved diagnostic tools for Pontiac fever (including molecular methods) that would enhance overall *Legionella* epidemiology and outbreak investigation and detection.

There is a need to better characterize legionellosis among neonates, young children, and adolescents, who may have varied epidemiologic risk factors for exposure to *Legionella* and differing risk for disease manifestations. There have been limited studies of Legionnaires' disease among children. The majority of current guidelines and recommendations focus on adult disease or target high-risk patient populations (e.g., immunocompromised hosts). Studies of community-acquired pneumonia,

fever, and non-respiratory viral influenza-like illness among pediatric populations that assess *Legionella* are needed.

Studies that further assess the contribution of aspiration of potable water as a mechanism for clinical legionellosis both in community outbreaks and among sporadic cases are needed. Inhaled respiratory droplets are thought to be the primary mode of exposure to *Legionella*, but other methods of exposure through water-based contact are poorly characterized. Aspiration, including microaspiration and silent aspiration, is thought to be potentially linked to legionellosis. Links between feeding tubes and among conditions that increase risk for aspiration, together with studies demonstrating potential short-term oral colonization, suggest potential risks from this pathway.

The capacity of *L. pneumophila* to resist detergents, heat, chemical disinfectants, and antibiotics, as well as predatory amoebae and white blood cells, depends on its growth phase. The resilience and infection potential differs by orders of magnitude for replicative, stationary or transmissive phase, and the mature infectious form of *L. pneumophila*. **Therefore, protocols should be developed to generate, identify, enumerate, and report distinct *Legionella* cell types.** These classifications are fundamental to not only experimental reproducibility but also to the development and accurate assessment of strategies to eliminate virulent legionellae from patients and engineered water systems.

Whether *L. pneumophila* persistence within built water systems is promoted by the bacterium's differentiation into an apparent viable-but-non-culturable state that is both resilient and reversible remains an urgent question with implications for public health. To date, studies of VBNC-like *L. pneumophila* are largely descriptive; for example, quantification of resuscitation from the VBNC-like form is lacking. Needed are protocols to generate and isolate pure populations of VBNC-like cells for physiological, biochemical, genetic, molecular, and infection studies. Molecular or biochemical markers that distinguish individual VBNC-like, MIF, replicating, and stationary phase *L. pneumophila* cells would also accelerate this research and enable experimentalists to distinguish true resuscitation of VBNC-like cells from regrowth of a minor population of cells.

Ecological studies have almost exclusively focused on the impact of environmental conditions on growth, survival, and inactivation of *L. pneumophila*. To clarify whether the ecological principles observed for *L. pneumophila* apply to other pathogenic *Legionella* species, research on the ecology of *L. longbeachae*, *L. micdadei*, *L. dumoffi* and other pathogenic *Legionella* species is warranted. In addition, studies on the ecology of *L. pneumophila* have focused primarily on building water systems, leaving the ecological conditions responsible for *L. pneumophila* growth in other environments (e.g., cooling towers, wastewater treatment plants, hot springs, soils) largely unexplored. New research in these two areas could result in improved control measures for other pathogenic *Legionella* species and for *L. pneumophila* in environments other than premise plumbing.

The acceleration of genomics in the clinical, environmental, and laboratory sciences has expanded awareness of the extraordinary diversity within the *Legionella* genus. Yet, most knowledge of *L. pneumophila* pathogenesis comes from a relatively small number of domesticated laboratory strains. **A wealth of genomic data can now inform research to identify specific genetic markers of resilience and virulence among the environmental and clinical *Legionella* and accelerate the rational design of risk assessment tools and environmental remediation methods.**

Clarifying the diversity of free-living protozoa that support or diminish the intracellular growth of legionellae in water and soil environments is fundamental to understanding and controlling legionellosis. Whether legionellae persist within free-living protozoa versus growing to high numbers appears to be influenced by many poorly understood factors, including temperature, species of bacterial prey available, presence of host symbionts, and host cell form. Direct (locational) observations and metagenomic studies of microbial diversity are required to provide a stronger ecological foundation to identify the protozoa that control the growth of pathogenic *Legionella* in various environments. Microcosm studies could investigate how nutrients and biocides affect the life stages of the host protozoa (e.g., by triggering encystation), identify the key host species, and elucidate the role of other free-living protozoa that might feed on the primary hosts of legionellae.

Despite clear evidence that people acquire Legionnaries' disease by inhaling contaminated water or soil, **neither the biochemical nor genetic attributes that equip *Legionella* bacteria to survive in aerosols have been identified.** Knowledge of the antigens or genetic loci that confer resilience of airborne legionellae and other factors like packaging (within biofilm, vesicles, trophozoites) would guide risk assessment of microbes that colonize engineered water systems.

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3
Quantification of Legionnaires' Disease and *Legionella*

This chapter addresses what is known about the incidence of Legionnaires' disease from surveillance systems and the occurrence of *Legionella* bacteria in water systems including the methods used to collect both clinical and environmental data. Both the tracking of disease incidence and monitoring the number of *Legionella* bacteria in various water systems are fraught with difficulties. These difficulties include deciding who to test, where and when to sample the environment, what methods to use, and how to interpret the data. Despite these challenges, advances have been made and are likely to continue as legionellosis becomes a higher public health priority.

Most cases of Legionnaires' disease are never linked to any specific environmental source, for many reasons. Most individuals are never diagnosed, even among those who seek medical care. Those who are diagnosed may have no associated clinical isolate to confirm the results of the urinary antigen test. Sampling for *Legionella* in buildings is routine in the United States for only a subset of acute care hospitals and other potential sources such as hotels. In addition, most states do not have the capacity to investigate environmental sources of Legionnaires' disease, with few environmental microbiologists or engineering experts on staff in public health departments. It is still the case that information on Legionnaires' disease stems mostly from investigations of recognized outbreaks, which account for only 4 percent of cases in the United States (Hicks et al., 2011). Not known is whether the environmental exposures found in outbreak investigations accurately represent the exposures for the majority of cases.

More information is needed about environmental exposures that result in disease in order to estimate their risk. To assess the level of risk of Legionnaires' disease, a quantitative microbial risk assessment (QMRA) framework can be designed using an estimate of the concentration of *Legionella pneumophila* (the pathogen most likely to cause disease) associated with a particular source (e.g., showerhead, hot tub, cooling tower) combined with dose-response information about the bacterium. As quantification of viable *Legionella* in water samples increases, this framework can be used to better understand which environmental exposures are most likely to lead to cases of legionellosis. This chapter ends with a discussion of the role of QMRA in linking clinical and environmental data and informing subsequent actions as well as in determining risk-based numerical values for *Legionella* in water.

INCIDENCE OF LEGIONELLOSIS IN THE UNITED STATES

To quantify Legionnaires' disease incidence, national surveillance is undertaken that builds on local and state surveillance efforts. All states require that public health authorities be notified of those diagnosed with Legionnaires' disease or Pontiac fever. In turn, states voluntarily report their numbers to the U.S. Centers for Disease Control and Prevention (CDC). Separately, states also report *waterborne disease* outbreaks to the CDC, including those caused by *Legionella*. Together this information serves as a basis for quantifying the incidence of Legionnaires' disease and contributes to our knowledge of the epidemiology of the disease. Before describing the nation's *Legionella* surveillance systems, the diagnostic tests used to identify cases of Legionnaires' disease are briefly reviewed (building on the Chapter 2 discussion).

Diagnostic Tests for Legionellosis Used in Surveillance

According to CDC, the preferred diagnostic tests for Legionnaires' disease are culture of lower respiratory secretions on selective media and the urinary antigen test. Serological assays can be nonspecific and are not recommended in most situations, while polymerase chain reaction (PCR) is utilized by some academic and reference laboratories.

Culture of sputum or bronchoalveolar lavage specimens from pneumonia patients is important to determine if *Legionella* is the causative agent, regardless of species and serogroup. *L. pneumophila* forms colonies on buffered charcoal yeast extract agar within three to five days. As discussed in Chapter 2, most non-*pneumophila Legionella* species (spp.) may require longer incubation times and different media, and some culture media do not support growth of certain non-*pneumophila Legionella* spp. Culturing *Legionella* is challenging because of the needs for a lower-respiratory specimen and technical expertise in the laboratory. Furthermore, a history of prior antibiotic use interferes with culture. Most hospitals do not routinely culture sputum for *Legionella,* although some academic health centers routinely culture bronchoscopy specimens in patients with pneumonia of unknown etiology. Culture methods are critically important to epidemiologic investigations because molecular analysis can link clinical isolates to environmental samples to document the source of the exposure.

Most patients with reported Legionnaires' disease are diagnosed as a result of a positive *Legionella* urinary antigen test (UAT), which is available at commercial laboratories. Its advantages include ease of use, relatively high sensitivity, and the ability to noninvasively diagnose *L. pneumophila* serogroup 1. The UAT also has a rapid turn-around time (within hours), but this benefit is only available at the 25 percent of acute-care hospitals that conduct the test on site; otherwise, one to three days or more are required (Garrison et al., 2014; McClean et al., 2010) or sometimes longer, particularly for sites that send samples to outside laboratories. The UAT's selectivity for *L. pneumophila* serogroup 1 means that patients with clinically important non-serogroup 1 *L. pneumophila* infections and non-*pneumophila Legionella* infections will be missed. Finally, as mentioned in Chapter 2, UAT results can be negative early in the disease course and are less likely to be positive with less severe disease (Mercante and Winchell, 2015).

Serology is a valuable tool for epidemiologic studies, but it has little clinical impact because of the delay in receiving results (Reller, 2003). Blood samples taken three to six weeks apart are analyzed for rises in antibody titer to *Legionella*. In most cases of Legionnaires' disease, a four-fold increase in antibody titer is detected within three to four weeks although it may take longer. Thus, both sensitivity and specificity of serologic tests can be problematic.

Molecular testing for *L. pneumophila* consists of highly sensitive PCR and other nucleic acid amplification tests. Most published studies utilize PCR testing that targets the macrophage infectivity potentiator (*mip*) surface protein of *L. pneumophila* (similar to the PCR tests done for environmental samples). As discussed in Chapter 2, PCR tends to detect more cases than UAT and culture tests, and it has the additional advantage of being useful in patients who are already on antibiotic therapy. PCR methods can currently detect *L. pneumophila* serogroup 1 and a few non-*pneumophila* species (Benitez and Winchell, 2013; Cross et al., 2016; Merault et al., 2011). Importantly, PCR for *Legionella* has been limited primarily to referral laboratories and research laboratories because of its difficulty, limited training, and the need for specialized instrumentation. Recently a multiplex PCR panel that includes *L. pneumophila* was approved by the U.S. Food and Drug Administration (FDA) for clinical use (Biofire® FilmArray® Pneumonia Panel) on sputum, endotracheal aspirates, bronchoalveolar (BAL), and mini-BAL lower-tract samples.

The criteria for diagnosing legionellosis used by the CDC are given in Box 3-1. These are likely to undergo revision in 2019 (Richard Danila, Minnesota Department of Public Health, personal communication, April 25, 2019).

Surveillance Systems for Legionnaires' Disease in the United States

All surveillance data must be interpreted in the context of the "surveillance steps" that lead to diagnosis and reporting (see Figure 3-1). To be counted as a case, a person with legionellosis must seek medical care or be assessed as part of an outbreak. A clinical specimen (e.g., urine, respiratory) must be submitted for testing, and the specimen must be tested for the presence of *Legionella*. This in turn requires that the laboratory be able to identify *Legionella*. All cases must meet the surveillance case definition given in Box 3-1. All 50 states, the District of Columbia, and U.S. territories (referred to collectively as the

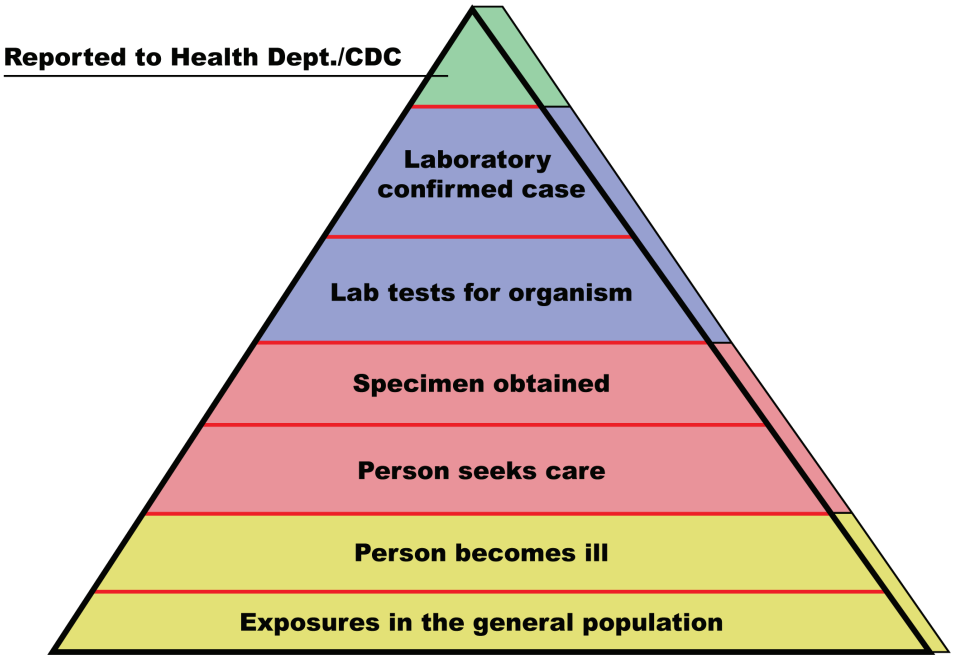

FIGURE 3-1 Disease surveillance steps.
SOURCE: Adapted from https://www.cdc.gov/foodnet/surveillance.html.

BOX 3-1
CDC Laboratory Criteria for Diagnosis of Legionellosis

Confirmed Cases:

- By culture: isolation of any *Legionella* organism from respiratory secretions, lung tissue, pleural fluid, or other normally sterile site
- By detection of *L. pneumophila* serogroup 1 antigen in urine using validated reagents
- By seroconversion: fourfold or greater rise in specific serum antibody titer to *L. pneumophila* serogroup 1 using validated reagents on specimens collected three to six weeks apart.

Suspected Cases:

- By seroconversion: fourfold or greater rise in antibody titer to specific species or serogroups of *Legionella* other than *L. pneumophila* serogroup 1 (e.g., *L. micdadei*, *L. pneumophila* serogroup 6) using validated reagents on specimens collected three to six weeks apart.
- By seroconversion: fourfold or greater rise in antibody titer to multiple species of *Legionella* using pooled antigen and validated reagents on specimens collected three to six weeks apart.
- By the detection of specific *Legionella* antigen or staining of the organism in respiratory secretions, lung tissue, or pleural fluid by direct fluorescent antibody (DFA) staining.
- By the detection of specific *Legionella* antigen or staining of the organism in respiratory secretions, lung tissue, or pleural fluid by immunohistochemistry (IHC).
- By detection of *Legionella* species by a validated nucleic acid assay (e.g., PCR).

SOURCE: CDC (2010).

states) require that cases diagnosed as Legionnaires' disease or Pontiac fever be reported to local or state public health authorities. These cases are to be reported from the state to the CDC. If any step in this process does not occur, an individual ill with legionellosis will not be counted by the CDC. When cases reported through surveillance are clustered in time and space, an outbreak may be identified.

As suggested in Figure 3-1, there are significant losses in numbers as one proceeds through the surveillance steps, such that the number of cases reported to the CDC is likely to be an underestimate of the true incidence of legionellosis by as much as eight- to ten-fold (Dooling et al., 2015; Mercante and Winchell, 2015; Phin et al., 2014; St-Martin et al., 2013; von Baum et al., 2008).

Two national surveillance systems maintained at the CDC have the capacity to collect information on all diagnosed cases of legionellosis from states. These are the National Notifiable Disease Surveillance System (NNDSS) and the Supplemental Legionnaires' Disease Surveillance System (SLDSS). Separately, CDC has regulatory authority over the cruise ship industry, which must report all cases of Legionnaires' disease to the CDC.

National Notifiable Disease Surveillance System

Since the disease's recognition in 1976, surveillance for legionellosis has been conducted by all states, the District of Columbia, and U.S. territories. Reporting is mandatory for all diagnosed cases of Legionnaires' disease and Pontiac fever by healthcare providers and clinical laboratories to local and state health officials; cases must be reported within a short time period from diagnosis, usually within one to seven days.

All cases of notifiable diseases are then reported voluntarily to CDC from public health officials in states through the National Notifiable Disease Surveillance System (NNDSS). Historically, notifiable diseases have been reported weekly, and the CDC has published preliminary case counts weekly. However, legionellosis reports are often sent to the CDC at irregular and sometimes lengthy intervals, such that the weekly counts may be low and the preliminary statistics for legionellosis often incomplete. Data shared on cases through this system are primarily demographic (e.g., place of residence) and clinical (e.g., date of onset of illness). Environmental source information, including the setting (e.g., hospital, hotel), type of water system (e.g., hot tub, decorative fountain), and type of water exposure (e.g., potable water, recreational untreated water) are not collected by the NNDSS. The NNDSS does not provide information on whether a case is travel-associated, healthcare-associated, or community-acquired.

Supplemental Legionnaires' Disease Surveillance System

A Supplemental Legionnaires' Disease Surveillance System (SLDSS) is available at the CDC to collect more comprehensive data on Legionnaires' disease cases from all states. The SLDSS includes potential environmental exposures, such as whether a case is travel-associated or whether an individual had exposure to hot tubs, respiratory therapy equipment, or a healthcare or senior-living facility. However, these data are often incomplete and not timely, and they frequently do not identify the potential environmental source of exposure. Therefore, these data have been insufficient to track trends in community-acquired, travel-associated, or healthcare-acquired cases (Cynthia Whitney, CDC, verbal communication, March 21, 2018).

In 2018, the CDC published the first surveillance summary focused on Legionnaires' disease using NNDSS and SLDSS data from 2014 and 2015, analyzing for associations with healthcare facilities, senior- or assisted-living facilities, and travel (CDC, 2018a). Future summaries are planned with the goal of better understanding the burden, impact, and trends of Legionnaires' disease over time.

Critique of National Surveillance and Next Steps

Given the loss of cases associated with each step in Figure 3-1, it is no surprise that the NNDSS and SLDSS do not account for most patients with legionellosis. In contrast to the steps leading to diagnosis, however, the reporting step itself is quite complete. In a 2011–2015 study conducted through the Active Bacterial Core Surveillance System to find all laboratory-confirmed cases of legionellosis, almost all cases found in the study had been previously reported through the NNDSS (Dooling et al., 2015).

Having two separate surveillance systems has been problematic, and the CDC plans to address the issue. The CDC is currently integrating the NNDSS and SLDSS through the NNDSS Modernization Initiative (Sam Posner, CDC, personal communication, September 21, 2018), a CDC-wide initiative designed to enhance the system's capabilities to provide more comprehensive, timely, and higher quality data. Case information that historically was sent through multiple routes will be consolidated into a single data stream.

Surveillance has been frequently referred to as "data for action," yet neither the NNDSS nor the SLDSS is robust for this purpose because states have not routinely investigated single cases for source(s) of exposure. Better understanding the source of environmental exposure could lead to improved prevention and control measures. Acknowledging that environmental investigation of every case is unlikely to occur because such investigations are resource intensive, more in-depth studies will be necessary to

investigate a subset of cases by setting, source of water (e.g., potable water supply, cooling tower), and building water system for potential environmental exposure.

For decades, legionellosis programs both in states and at the CDC have been given low priority compared to other preventable infectious diseases, including communicable respiratory conditions. Furthermore, because the programs were initially focused on outbreak detection and control, the CDC and other public health agencies did not build expertise and capacity in fields that are needed to understand legionellosis prevention and control (e.g., building water systems, environmental engineering, and industrial hygiene). Legionellosis surveillance has not had dedicated resources to ensure timely environmental investigation of cases. Many state public health laboratories do not have the resources to identify, quantify, or subtype *Legionella* in water specimens; only three states have capacity to perform genome sequencing (Richard Danila, Minnesota Department of Health, email communication, September 29, 2018). CDC has recently devoted resources to legionellosis in some states through its Epidemiology and Laboratory Capacity cooperative agreements. These include Arizona, California, Colorado, Georgia, Illinois, Los Angeles County, Maryland, Michigan, Minnesota, Nebraska, Nevada, New York City and State, Ohio, Philadelphia, Tennessee, Utah, Virginia, Washington, DC, and Washington State. Some agreements have focused on getting public health laboratories, environmental health experts, and epidemiologists working together; others emphasize locating, registering, and testing cooling towers, whereas others focus on hotels; still others prioritize better cluster detection (Richard Danila, Minnesota Department of Health, personal communication, July 23, 2018). More efforts like these cooperative agreements are needed to help state and local health departments build their capacity for *Legionella* surveillance and response. New York City provides one of the most comprehensive legionellosis surveillance systems in the United States (see Box 3-2).

With respect to travel-associated cases, the Council of State and Territorial Epidemiologists (CSTE) has stated that surveillance for legionellosis lacks the timeliness and sensitivity necessary to detect outbreaks of these cases (CSTE, 2005). CDC is uniquely positioned to identify connections between cases that occur in residents of different jurisdictions, which is most likely with travel-associated outbreaks. It is particularly important that travel-associated cases be reported by the states to the CDC in almost real time to prevent delays in investigation and control. Following the 2005 CSTE position statement, CDC instituted a dedicated email address to improve reporting of travel-associated cases. Europe has a more extensive reporting system for travel-associated cases, discussed later in this chapter.

Academic centers currently play little, if any, role in either building or assessing prevention and control efforts for legionellosis. If the CDC chose to take a much more comprehensive approach to legionellosis, both the Integrated Food Safety Centers of Excellence and the Regional Centers of Excellence in Vector-Borne Diseases could serve as models. Under the Food Safety Modernization Act of 2011, the CDC designated six Integrated Food Safety Centers of Excellence at state health departments and affiliated university partners not only to identify and implement best practices in foodborne disease surveillance and outbreak response, but also to serve as a resource for other state, regional, and local public health professionals[1]. In 2017, five universities were established as regional Centers of Excellence to help prevent and rapidly respond to emerging vector-borne diseases across the United States. The goals of these centers include building effective collaboration between academic communities and public health organizations at federal, state, and local levels for surveillance, prevention, and response; training public health experts in the knowledge and skills required to address vector-borne disease concerns; and conducting applied research to develop and validate effective prevention and control tools and methods and to anticipate and respond to disease outbreaks.

[1] See https://www.cdc.gov/foodsafety/centers/index.html, accessed March 9, 2019.

BOX 3-2
Legionellosis Surveillance Data Summary, New York City, 2007–2017

Surveillance Methods

New York City (NYC) legionellosis surveillance data are comprised of reported positive *Legionella* clinical laboratory test results, clinical patient information, and patient exposure information obtained through patient interview. The NYC Health Code mandates that positive *Legionella* clinical laboratory test results be reported to the NYC Department of Health and Mental Hygiene (DOHMH). Electronic laboratory reports are sent to the NYC DOHMH Bureau of Communicable Disease through the Electronic Clinical Laboratory Reporting System. For each reported positive *Legionella* clinical laboratory test (urinary antigen test, culture, PCR, or paired serology) the NYC DOHMH conducts: (1) a medical record review using a standardized data abstraction tool; and (2) a standardized 11-page telephone or in-person interview of the patient or their next-of-kin. The healthcare facility's medical records include chest x-ray and computed tomography (CT) scan results, along with the recorded history of the patient's clinical symptoms and medical treatment. The patient interview collects information on the patient's home, work, and other addresses, presenting symptoms, and health history, along with information on known water exposures, travel, and healthcare visits during the ten days before onset of symptoms (the typical disease incubation period). Information gathered from these sources is used to determine if the patient's illness meets the case definition of a confirmed or possible case of legionellosis, and to assess if there are possible exposure sources or locations that require further investigation, based on the occurrence of legionellosis among other people who shared those possible exposures.

Results of Trends in Reported Legionellosis Cases, NYC, 2007-2017

As shown in Figure 3-2-1, from 2007 to 2017 rates of legionellosis increased for both men and women, and in all age groups. Legionellosis cases occurred more frequently among men (62 percent) than women (38 percent). The majority (69 percent) of patients diagnosed with legionellosis were adults aged 55 years or older. Rates of legionellosis increased for all racial groups, with the highest rate of increase among the non-Hispanic Black/African American population. Thirty nine percent of all cases occurred in people who identified as non-Hispanic Black/African American (approximately 22 percent of New Yorkers are non-Hispanic Black/African American). Rates of legionellosis increased in all five NYC boroughs. The largest number of legionellosis cases (n = 472, 32 percent of all cases) occurred in the Bronx, home to about 17 percent of the NYC population. Rates of legionellosis increased in neighborhoods of all income levels. The highest rates and the greatest rate of increase occurred in very high poverty neighborhoods.

Figure. Trends in Legionnaires' disease rates per 100,000 people, by demographic groups, NYC 2007-2017

Group	2007	2017	AAPC	*p*-value
Total	2.3	5.1	8.1	<0.01
Sex				
Female	1.4	3.8	9.2	<0.01
Male	3.2	6.5	7.4	0.01
Race and Ethnicity				
Black/African American (NH)	2.5	7.6	11.4	<0.01
Latino/Hispanic	1.6	3.3	8.3	<0.01
White (NH)	2.2	5.4	8.3	0.01
Asian (NH)	0.4	2.4	21.1	<0.01
Age Group (years)				
<35 years	0.2	1.6	9.3	0.03
35 to 54 years	1.5	2.5	4.4	0.11
55 to 64 years	4.0	8.6	9.1	<0.01
65 to 74 years	3.2	10.1	11.6	<0.01
75 to 84 years	6.1	11.0	6.2	0.01
≥85 years	5.2	10.2	7.9	0.03

Group	2007	2017	AAPC	*p*-value
Borough of Residence				
Bronx	4.1	7.0	6.6	0.02
Brooklyn	1.8	3.4	4.8	0.02
Manhattan	2.7	6.4	9.4	0.01
Queens	1.5	4.8	11.0	<0.01
Staten Island	1.5	5.7	15.7	<0.01
Neighborhood Poverty Level (% below FPL)				
Low (<10%)	2.0	4.8	7.3	0.02
Medium (10 to <20%)	1.6	4.5	9.2	<0.01
High (20 to <30%)	2.3	4.6	5.7	0.03
Very High (≥ 30%)	3.2	6.4	9.6	<0.01

Abbreviations: AAPC, average annual percent change; FPL, federal poverty level; NH, non-Hispanic.
Data sources: Population and poverty data from NYC DOHMH population estimates, modified from US Census Bureau intercensal population estimates, 2000-2016. Updated September 2017.

FIGURE 3-2-1 Trends in Legionnaires' disease rates per 100,000 people.

Results from Medical Record and Patient Interview Data, NYC, 2013-2017

Health Conditions and Behaviors. The majority of legionellosis patients (72 percent) reported at least one chronic health condition. The most common conditions reported were diabetes (24 percent) and lung disease (19 percent). About half (45 percent) of patients reported a history of current or past tobacco smoking.

Exposure Settings. About 8 percent of legionellosis cases were definite healthcare-associated,[1] while about 4 percent were possible healthcare-associated.[2] About 9 percent of legionellosis patients reported traveling outside of NYC for at least one day during their ten-day disease incubation period. Among people diagnosed with legionellosis, 23 percent reported working during their incubation period.

Reported Water Exposures or Changes to Water Service. The following possible water exposures were reported by legionellosis patients as occurring during the ten-day disease incubation period: air humidifier, 1 percent; hot tub, 1 percent; swimming pool, 1 percent; decorative fountain, 2 percent; gym, 2 percent; respiratory equipment, 5 percent; shower outside home, 5 percent; grocery store, 10 percent. In 5 percent of cases, patients reported plumbing maintenance at the residence during the ten-day disease incubation period, and in 5 percent of the cases, patients reported a water service disruption.

Clinical Diagnostic Testing

The majority (90 percent) of legionellosis cases were diagnosed by *Legionella* urinary antigen test only. Ten (10) percent of legionellosis cases included an isolate from a clinical culture that could undergo molecular analysis for comparison to isolates from possible environmental sources.

Conclusions

From 2007 to 2017, legionellosis in NYC occurred at the highest rates among those who were aged 55 years and older, in neighborhoods with the highest poverty rates, and among those who identified as non-Hispanic Black/African American. From 2013 to 2017, the majority of people diagnosed with legionellosis in NYC had chronic conditions or health behaviors that are reported risk factors for developing legionellosis, including diabetes, chronic lung disease, and tobacco smoking. Data from patient interviews and medical record reviews point to the challenges involved in using surveillance data to identify a source for individual cases of legionellosis that are not part of a cluster: nearly 90 percent of cases were community-associated, where numerous exposures to aerosols of water may occur during the ten-day disease incubation period. Only a very small proportion of people recall specific water exposures during their ten-day disease incubation periods. Conversely, many people may experience unrecognized aerosol exposures during that time, from cooling towers and other sources. These patient histories offered little guidance for testing possible environmental sources for individual cases of legionellosis.

Only about 10 percent of cases included a clinical isolate that can undergo molecular analysis for comparison to isolates from possible environmental sources. Thus, NYC's experience suggests that even if local and state health departments had budgetary and personnel capacity to test any and all possible environmental exposures for each individual case of legionellosis, source attribution would be possible, at best, for only about 10 percent of cases.

These data indicate that any rigorous effort to better understand the sources of exposure that cause individual legionellosis cases will require well-funded, coordinated studies involving medical centers, laboratories, and health departments in areas with capacity for the consistent collection and cultivation of both clinical and environmental *Legionella* cultures for a substantial proportion of cases. This is resource intensive because most sporadic cases involve multiple possible environmental sources of *Legionella* exposure, and environmental isolates that do not match clinical isolates may still require on-going public health follow-up when they indicate possible disease risk from a potential environmental source.

[1] Patient spent all of the ten-day disease incubation period in an acute-care hospital or nursing home.
[2] Patient spent some portion of the ten-day disease incubation period in an acute-care hospital or nursing home.

U.S. Department of Veterans Affairs Surveillance System

In addition to the national systems, the Veterans Health Administration (VHA) collects information on all cases of legionellosis within its healthcare system. The VHA operates the largest integrated healthcare system in the United States, with more than 1,200 sites of care, serving about 6 million veterans annually. In federal fiscal year (FY) 2016, 91 percent of veterans using VHA benefits were male, with a median age of 64 years and with higher morbidity than in the rest of the United States (Gamage et al., 2018), which as discussed in Chapter 2 are populations with an increased risk of contracting Legionnaires' disease. As discussed in detail in Chapter 5, the VHA has a *Legionella* prevention policy for medical facilities to limit *Legionella* growth in building water systems, requiring the collection of both environmental and clinical data. Concomitant to publication of the policy in 2014, the VHA Central Office implemented a national standardized Legionnaires' disease reporting system. Compared to the CDC's notifiable disease reporting system, the VHA collects more detailed information on each case, partly to assess if a person was exposed while inside a VHA facility. As more environmental data are collected throughout the VHA system, the surveillance system will become critical for evaluating the effectiveness of the VHA's legionellosis prevention policies and also provide useful information for public health agencies and other healthcare facilities.

Waterborne Disease Outbreak Reporting System of the National Outbreak Reporting System

A third U.S. national surveillance system—the National Outbreak Reporting System or NORS—is also maintained by the CDC and collects data on waterborne and foodborne disease outbreaks in the United States. CDC categorizes the sources of waterborne disease outbreaks as follows: (1) drinking water, (2) treated recreational water, (3) untreated recreational water, and (4) another environmental exposure or undetermined source. *Legionella* was added to this system in 2001. Data from this system are currently publicly available on the NORS dashboard;[2] one can sort outbreaks by etiologic agent, year, state, setting (e.g., hotel, trailer park, hospital), water exposure (see above), and type of water system (e.g., hot tub, decorative fountain, cooling tower). NORS does not include detailed information on the setting and type of water system, which would be particularly useful for improving understanding of sources and conditions conducive to transmitting legionellosis.

The waterborne disease outbreak reporting system was initiated in 1971 as a partnership between CDC, CSTE, and the U.S. Environmental Protection Agency (EPA). It is dependent on public health departments in individual states to voluntarily provide complete and accurate data for waterborne disease outbreaks. The waterborne disease outbreak reporting system is important because outbreaks are most likely to be investigated for environmental sources.

A limitation of the NORS program for legionellosis is that the database (and hence the categories of setting, water types, and water exposure) was developed for enteric pathogens, making it less useful for pathogens capable of growth in water systems and transmitted by aerosolized water. Also, NORS data for legionellosis are not updated frequently; until December 2018, only data through 2014 were available.

[2] See www.cdc.gov/norsdashboard.

European Surveillance

In most European countries, laboratory-confirmed Legionnaires' disease cases must be reported to the public health authorities of the country (e.g., in Germany, reporting is mandatory to national authorities within 24 hours of diagnosis). Most countries of the European Union report annually to the European Centers for Disease Control (ECDC) through the European Legionnaires' Disease Surveillance Network (ELDSNet) (Lara Payne, ECDC, personal communication, October 6, 2018). In 2017, 30 countries participated in ELDSNet. Members of this network review relevant technical documents and assist ECDC in organizing an annual meeting. ELDSNet collaborates with partners, such as the World Health Organization (WHO), public health authorities of non-EU countries, and tour operators. The incidence of Legionnaires' disease in Europe ranges widely among countries, which may largely reflect the variability in diagnosis and reporting. The burden of disease and trends are analyzed and reported in a detailed annual surveillance summary dedicated to Legionnaires' disease (e.g., ECDC, 2019).

Considerable focus of ELDSNet has been on travel-associated Legionnaires' disease, which accounts for approximately 20 percent of cases. (The European definition of travel-associated is more restrictive than in the United States and requires a stay in an overnight accommodation in the ten days before symptom onset.) The operating procedures of the surveillance scheme for travel-associated Legionnaires' disease in the EU and European Economic Areas (EEA) were updated in December 2017 (ECDC, 2017a), such that these cases are reported in almost real-time. In 2015, the estimated median delay between onset of illness and report to ELDSNet was only 17 days. When a cluster is identified within an EU/EEA country, all participating countries are notified and the public health authorities where the accommodation site is located are expected to report on the investigations conducted on the accommodation site. If the timeline for reporting is not fulfilled or control measures are deemed unsatisfactory by the ECDC, the name of the accommodation site is published on the ECDC website and the International Federation of Tour Operators is notified.

Trends in Reported Legionellosis in the United States

From 2007 to 2017, the rate of reported legionellosis cases through the NNDSS increased from 0.91 cases to 2.29 cases/100,000 persons, with more than 7,400 cases reported in 2017. Although case reporting is officially for legionellosis, 98 percent of the case reports represent individuals hospitalized with pneumonia (Dooling et al., 2015). Therefore, the trends primarily reflect more severe cases of Legionnaires' disease. It is likely that trends in treatment of outpatients with Legionnaires' disease and Pontiac fever follow trends similar to the hospitalization data.

Reported rates of legionellosis are lower in some areas of the United States (e.g., the West) than other areas. But for all areas of the country, the rates have increased from 2005 to 2015 (see Figure 3-2; Cooley, 2018). Weather patterns likely contribute to geographic differences, with warm, humid weather increasing Legionnaires' disease risk. Population and building density as well as regional differences in water treatment could also be playing a role.

In the United States, seasonal trends are evident, with cases rising in late spring, increasing in the summer, and peaking in late summer and fall. In 2016, 78 percent of cases were reported for the seven months of June through December. The lowest months are generally January through April. As with other variables, for all months from 2007 to 2016, the trend in incidence is generally upward.

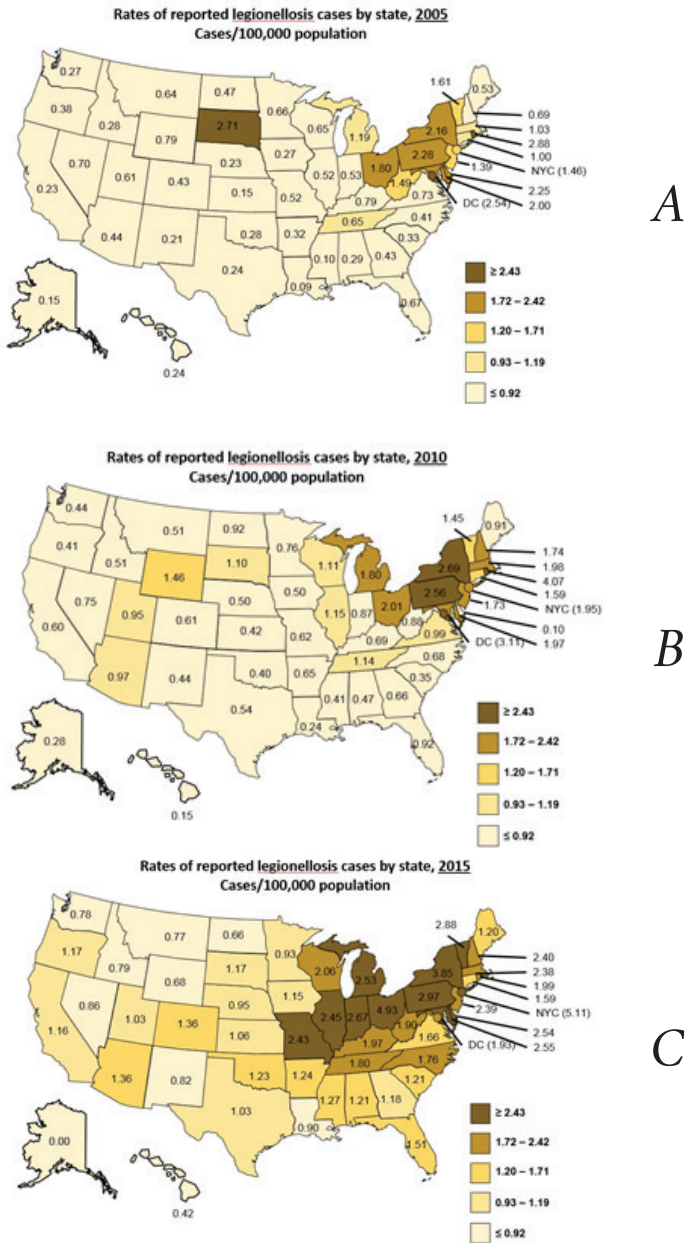

FIGURE 3-2 Rates of reported legionellosis cases by state for 2005 (A), 2010 (B), and 2015 (C). Values are cases per 100,000 population. SOURCE: Cooley (2018).

After leveling off or decreasing from 2007 to 2010, European case rates have increased from 1.0 to 1.8 cases/100,000 persons from 2011 to 2017 (see Figure 3-3), with the majority of cases (69 percent) reported from France, Germany, Italy and Spain. Australia has also noted increases in cases of *L. pneumophila* between 2005 and 2014 but not of *Legionella longbeacheae. L. longbeacheae* disease is rarely reported in the Unites States. Figure 3-3 shows that European rates are slowing relative to those of the Unites States, with the U.S. rate superseding the European rate since 2011.

Legionellosis cases can be subdivided into various categories. For example, cases may be recognized as part of an outbreak, a term used to describe two or more people with Legionnaires' disease exposed to *Legionella* at the same place at about the same time. Cases not recognized as part of an outbreak

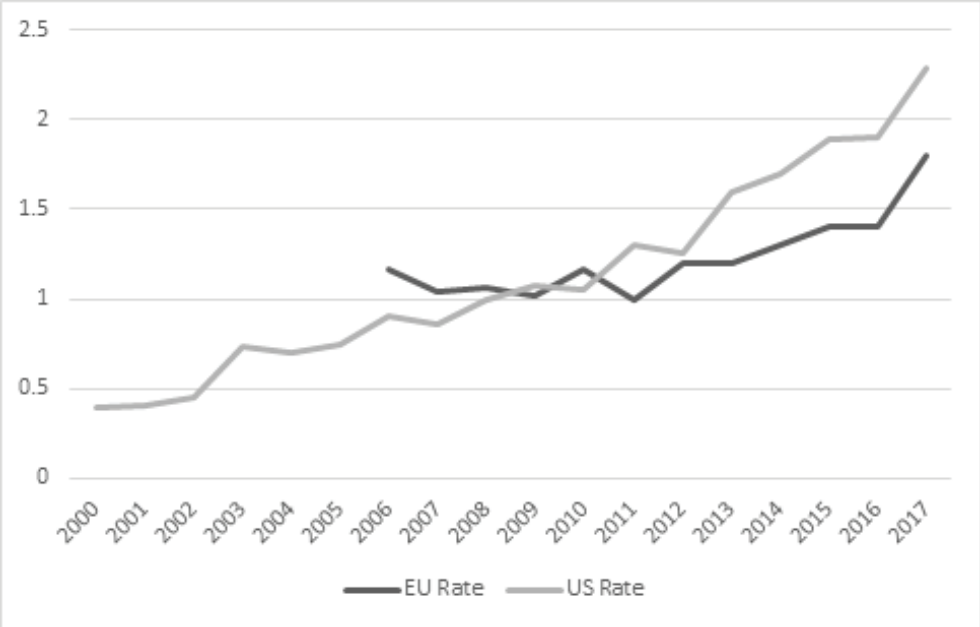

FIGURE 3-3 U.S. and European trends in Legionnaires' disease rate (number per 100,000 people).
SOURCES: 2013–2017 European data from ECDC (2019); 2012 European data from ECDC (2018); 2011 European data from ECDC (2017b); 2008–2010 European data from ECDC (2016); 2006–2007 European data from ECDC (2014); 2000–2009 U.S. data from Hicks et al. (2011); 2010–2015 U.S. data estimated by the Committee from https://www.cdc.gov/legionella/qa-media.html; 2016 U.S. data from CDC (2017b); 2017 U.S. data from CDC (2018b).

are considered sporadic. In the United States, waterborne disease outbreaks in the NORS system are subdivided into whether the outbreak source was identified as potable water, recreational water (treated or untreated), or another water source.

Frequently, cases are also categorized as either "healthcare-associated," "travel-associated," or "community-acquired." "Definite" healthcare-associated cases are defined as patients that stayed overnight in a healthcare facility (e.g., a hospital or long-term care facility) for the entire ten days before symptom onset, while "possible" cases are defined as patients with exposure to a healthcare facility for a portion of the ten days preceding symptom onset (CDC, 2018a). Travel-associated cases must have a history of spending at least one night away from home, either domestically or abroad, in the ten days before symptom onset (CSTE, 2005). Cases are designated as community acquired when the patient did not spend at least one night away from home in the ten days before onset of illness or was not exposed to a healthcare facility in the ten days before onset of symptoms. Various categorizations are used below to parse occurrence data in the Unites States.

Healthcare-Associated Cases

Healthcare-associated cases of Legionnaires' disease make up approximately 20 percent of all legionellosis cases reported in the United States. In 2015, among 21 jurisdictions that reported exposure information on more than 90 percent of cases through the SLDSS, 3 percent of cases were considered "definite" and 17 percent had "possible" exposure to a healthcare facility in the ten days before symptom onset (Soda et al., 2017). Of the definite cases, 80 percent were associated with long-term care facilities,

18 percent with hospitals, and 2 percent with both. In addition, 3 percent were associated with assisted- or senior-living facilities (CDC, 2018a). An analysis of case reports to the ECDC between 2011 and 2015 reported 7.3 percent as healthcare-related, 4.9 percent of cases as nosocomial (i.e., from a hospital specifically) and 2.4 percent as "other" healthcare-related cases (Beauté, 2017).

Data from the VHA between 2014 and 2016 show that the rate of Legionnaires' disease significantly increased among veterans receiving VHA healthcare services but with no exposure to a VHA healthcare facility during the disease incubation period (from 0.9 to 1.47/100,000 enrollees). The rate of Legionnaires' disease among those with an overnight stay at a VHA facility during the disease incubation period significantly decreased (from 5.0 to 2.3/100,000 enrollees with an overnight stay). Most "definite" cases of healthcare-associated Legionnaires' disease (11 of 13) were in long-term care VHA facilities (Gamage et al., 2018).

Travel-Associated Cases

The CDC has reported data on travel-associated Legionnaires' disease from a limited number of jurisdictions. Benin (2002) found that 20 percent of Legionnaires' disease cases were reported as possibly travel-associated between 1980 and 1998. From 2005 to 2006, 24 percent of cases reported through the SLDSS were possibly travel-associated (Smith et al., 2007).

In Europe, 20 percent of Legionnaires' disease cases reported between 2011 and 2015 were travel-associated (Beauté, 2017). ECDC's case definition for travel-associated cases includes only lodging in a commercial establishment (e.g., hotel, resort), which is a more restrictive definition than the U.S. definition, in which any night away from home during the incubation period was reported as travel-associated. Nonetheless, data on travel-associated cases in the United States are similar to European data.

Box 3-3 discusses Legionnaires' disease rates for cruise ships, which have plateaued. Hotels and other commercial accommodation sites have been clearly documented to be an important source of environmental exposure to *Legionella.*

BOX 3-3
Cruise Ship Industry: Legionnaires' Disease Prevention and Control Efforts, 2007–2017

Despite a 21 percent increase in passengers and a marked increase in Legionnaires' disease outbreaks in the United States in the past decade, there was no significant increase in cruise ship associated outbreaks reported to the CDC in the five-year period from 2007 to 2011 (11 outbreaks) compared to 2012 to 2016 (12 outbreaks). In 2017, there were two cruise ship outbreaks (Sam Posner, CDC, email communication, April 17, 2019). These data suggest that prevention measures taken by the cruise ship industry appear to have been at least partially effective in addressing the threat of Legionnaires' disease outbreaks associated with cruise travel. Many in the cruise ship industry have engaged *Legionella* consultants to assure safety of their water supply and have conducted routine environmental sampling for *Legionella*, including quantitative culturing. Of particular note, CDC has regulatory authority over vessel sanitation and has provided guidance to cruise ships for Legionnaires' disease prevention for more than 20 years. The guidance and inspections of hot tubs and other potential environmental sources by the CDC's Vessel Sanitation Program (VSP) and the adverse publicity and liability associated with outbreaks investigated by CDC using its regulatory authority may have contributed to the attentiveness of cruise lines to maintenance of their water operations. The VSP 2011 Operations Manual and updates are available at http://www.cdc.gov/nceh/vsp.

Community-Acquired Cases

Most Legionnaires' disease cases in the United States are considered to be community-acquired (either sporadic or as part of an outbreak). This is consistent with what is found in Europe, where 70 percent of Legionnaires' disease cases reported to ELDSNet between 2011 and 2015 were community-acquired (Beauté, 2017). Similarly, the Robert Koch Institute (2013, 2015) estimated that about 70 percent of reported legionellosis cases are neither related to an outbreak nor nosocomial, but rather acquired in private or professional surroundings.

Unfortunately, most of the information on community-acquired cases in the United States comes from outbreak investigations or from the many publications on individual outbreaks. The most comprehensive review of sporadic, community-acquired cases (Orkis, 2018) included 47 articles on sporadic cases (excluding healthcare- and outbreak-associated cases) in which a total of 28 environmental sources were identified. Potable water from single family homes, large building water systems, and car travel appeared to contribute to a substantial proportion of the sporadic Legionnaires' disease cases. Cooling towers were also noted to be a potentially significant source. The difficulty in source attribution was noted, with definitive links using molecular typing between environmental sources and clinical isolates being made in only eight cases. The authors noted that understanding the risk magnitude of potential sources would make future public health investigations more efficient and enhance prevention efforts.

den Boer (2015) performed source investigations on more than 75 percent of 1,991 patients with Legionnaires' disease between 2002 and 2012 (source investigations were only done for clusters of disease after 2006). The paper noted the difficulty and the resource intensity of investigations to locate with certainty the source of an infection, and it reported outcomes of investigations of sporadic cases together with outcomes of cluster investigations. Of the 1,484 source investigations performed, only 367 (24.7 percent) of the sources were positive for *Legionella* spp., and only 41 patients (2.3 percent) were found to have a clinical strain that matched the environmental source. The sources that matched included a healthcare setting (40 percent), residence (18 percent), industrial complex (8 percent), swimming pool (5 percent), wellness center (8 percent), hotel (5 percent), spa (5 percent), and car wash (3 percent). The study also examined 105 clusters associated with 266 patients based on location and geography: 26 percent of the clusters were associated with garden centers, 16 percent with healthcare facilities, 10 percent with a residence, 9 percent with wellness centers (e.g., spas, saunas), 7 percent with hotels, 5 percent with cooling towers, and 5 percent with holiday parks.

Che and colleagues (2003) reported an increased risk of sporadic cases of community-acquired Legionnaires' disease in industrial areas of France. They evaluated 880 cases from 1998 to 2000 that were not associated with an outbreak and in which individuals did not report an overnight hospital stay or traveling within ten days of disease onset. Seventy-nine percent of the cases were caused by *L. pneumophila* serogroup 1. A higher risk was reported in areas with exposure to aerosols and plumes of smoke, with the greatest risk being in areas with more than one industrial exposure. However, the results are inconclusive and the findings deserve further study.

A study by the New York City (NYC) Department of Health and Mental Hygiene looked at the potential role of occupation among 335 community-acquired cases. Compared with the general population, legionellosis case-patients who were working in the two weeks before diagnosis were significantly more likely to work in transportation, repair, protective services; cleaning services; or construction (Farnham et al., 2014).

Community-acquired cases are commonly attributed to private water systems, under the assumption that the small number of people exposed would not draw the attention of epidemiologists to investigate. For example, Bonilla Escobar et al. (2014) demonstrated that a healthy, immunocompetent

young person with no other risk factors contracted Legionnaires' disease from an improperly maintained household humidifier, but no conclusions were drawn about the frequency of humidifiers being sources of *Legionella* infections. In another case study, two unrelated individuals appeared to contract Legionnaires' disease in their homes and both had solar water heaters with inadequately heated water (Erdogan and Arslan, 2016). Currently, it is largely unknown how often private water sources, particularly in individual homes, are the environmental exposure source for sporadic cases.

Outbreak Data That Reveal Environmental Sources

Most legionellosis outbreaks are detected through analysis of surveillance data compiled through the mandatory reporting systems described above. As discussed previously, and unlike the surveillance data reported through NNDSS or SSLDS, NORS data (now available from 2009 to 2017) are examined by water type, i.e., whether the outbreak is associated with drinking water, treated or untreated recreational water, or another water system. During 2013 to 2014, 19 states reported 42 outbreaks associated with **drinking water;** *Legionella* was implicated in 57 percent of the outbreaks (see Figure 3-4), 13 percent of the cases, 88 percent of the hospitalizations, and all 13 deaths[3]. From 2000 to 2014, NORS reported 363 outbreaks associated with **treated recreational water** that had a confirmed infectious etiology; 16 percent were caused by *Legionella* and legionellosis was confirmed or suspected to be responsible for all eight deaths (Hlavsa, 2018). During 2013 to 2014, 15 outbreaks were associated with "**another" environmental exposure to water**; *Legionella* was responsible for 63 percent of the outbreaks, 94 percent of hospitalizations, and all 17 deaths (McClung et al., 2017). Finally, 11 of 12 outbreaks associated with an **undetermined exposure to water** were caused by *Legionella* (McClung et al., 2017).

[3] See www.cdc.gov/healthywater/surveillance/drinking-water-tables-figures/html.

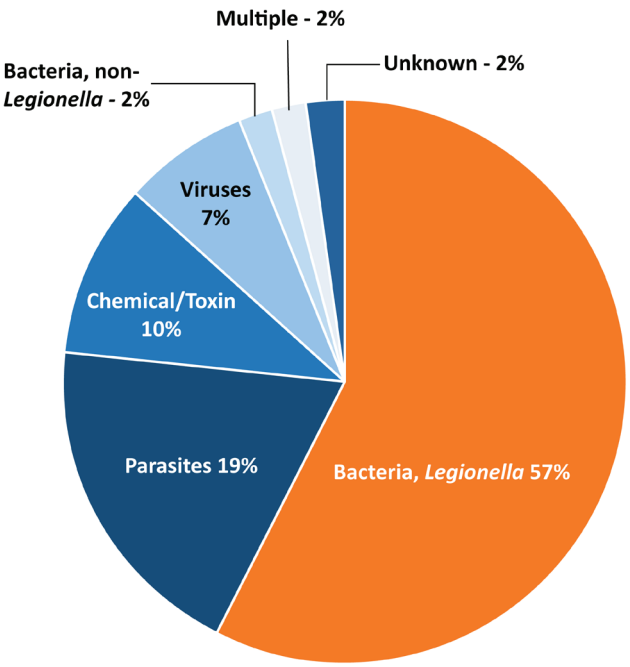

FIGURE 3-4 NORS reported drinking water-associated disease outbreaks, 2013–2014 (n=42).

SOURCE: CDC (2017).

Unfortunately, published analyses of NORS data generally do not reveal the setting (e.g., hotel, hospital) or water exposure (e.g., spa, decorative fountain), although some of the data are available and could be stratified for further analysis. The Committee analyzed NORS data between 2009 and 2017, during which 290 legionellosis outbreaks were reported. A substantial percentage of cases were associated with hotels and healthcare facilities. Other implicated locales included long-term care facilities, assisted-living or rehabilitation facilities, apartment buildings, indoor workplaces, factories or industrial settings, and prisons. Within those settings, cooling towers, hot tubs, and ornamental fountains were implicated. The goal of this cursory analysis is to raise awareness of the data available via the NORS dashboard that could be analyzed to determine environmental exposures associated with legionellosis cases.

Garrison and colleagues (2016) analyzed data from 27 building-associated Legionnaires' disease outbreaks (2000–2014) that were investigated by the CDC between 2000 and 2014. Common exposure settings were hotels (44 percent), long-term care facilities (19 percent), and hospitals (15 percent). Common sources (within the settings) were found to be showers and faucets (56 percent), cooling towers (22 percent), hot tubs (7 percent), decorative fountains (4 percent), and industrial equipment (4 percent).

By reviewing the peer-reviewed literature and government documents published between 2006 and 2017, Hamilton and colleagues (2018a) identified 119 legionellosis outbreaks globally for which an environmental source was associated with the event. Potable water was identified as the source in 42 outbreaks (30 percent), although this was not subdivided to better understand whether a specific water system or fixture deficiency was the culprit. Cooling towers, air conditioning, or evaporative condensers were identified in 41 outbreaks (30 percent). Cooling towers were associated with 50 percent of the confirmed cases of legionellosis and the greatest number of fatalities. Fifteen (15) percent of outbreaks occurred at hotels.

One of the world's largest outbreaks of Legionnaires' disease was linked to a hot tub exhibited at a Dutch flower show (den Boer et al., 2002). Simply pausing at the hot tub was deemed the most important risk factor for infection, confirming that a contaminated hot tub, even if not used directly, can cause illness in susceptible people. Of particular importance is the potential role of municipal water systems. In Flint, Michigan the governor's task force concluded that the management of the Flint River-sourced water supply may have contributed to the outbreaks of legionellosis in 2014 and 2015 in Genesee County (Flint Water Advisory Task Force, 2016), and scientific studies identified aspects of the water that were conducive to *Legionella* proliferation (Rhoads et al., 2017; Zahran et al., 2018). Outbreaks have also been attributed to wastewater treatment plants (Kusnetsov, 2010; Loenenbach et al., 2018).

The investigation of a large outbreak of Legionnaires' disease in NYC in 2015 illustrates how a multi-disciplinary approach to outbreak detection and subsequent investigation can lead to successful control (Box 3-4, Chamberlain, 2017). This investigation is unique in its scope, timeliness, and the extent to which clinical and environmental data were paired to determine the source of the *Legionella.* It also illustrates the resource intensity and difficulty of investigations of Legionnaires' disease outbreaks.

Why Are Rates of Legionnaires' Disease Increasing?

Although often put forward as potential explanations for the increase in Legionnaires' disease incidence, neither improved reporting nor improved diagnosis are supported by available data as a major contributor to the rapid increase since 2000. Indeed, reporting of diagnosed cases was documented to be extremely high for the period 2011 to 2013 (Dooling et al., 2015). Currently there are very limited data available to assess the role of diagnostic testing in increased incidence.

BOX 3-4
2015 Legionnaires' Disease Outbreak Investigation, Bronx, New York

In July 2015, the New York City Department of Health and Mental Hygiene (DOHMH) detected an increase in cases of Legionnaires' disease in the South Bronx, using the surveillance system described in Box 3-2. The purpose of the investigation was to describe patient demographic characteristics and comorbidities, identify environmental exposures, and implement control measures.

Reporting and Case Follow-up

Physicians and clinical laboratories are required to report positive *Legionella* test results to DOHMH. For each case reported, epidemiologists review patient medical records, interview the patient (or the patient's proxy) to determine if the report meets the CSTE/CDC national case definition for legionellosis, and identify risk factors and potential exposures.

Cluster Identification Analyses

Two methods are used to identify clusters that could be community outbreaks of reportable diseases. Each week, the historical limits method compares case volume in the most recent four-week period with comparable data from the previous five years at the city, borough, and neighborhood levels. A separate daily spatiotemporal cluster detection method is based on the space-time permutation scan statistic, and computes a ''recurrence interval,'' which is the number of days of surveillance required for the expected number of clusters at least as unusual as the observed cluster to be equal to 1 by chance. Additionally, an automated daily algorithm compares the building identification number (i.e., a unique code for every structure in New York City) assigned to the patient's address with a list of health care and congregate living facilities to identify concerning events not already detected by epidemiologists.

To guide environmental sampling, a multi-focused cluster test with the space-time permutation scan statistic was used to assess clustering of cases of Legionnaires' disease around cooling towers.

Case Definitions

An outbreak-associated case of Legionnaires' disease was defined as clinically compatible illness meeting the national case definition for Legionnaires' disease (Box 3-1), modified to include *L. pneumophila* serogroup 1 (Lp1) DNA detected by quantitative PCR (qPCR) in postmortem specimens, in either a resident of one of seven South Bronx ZIP codes (i.e., the outbreak zone) or in a person who worked in or visited the outbreak zone during the ten days before his or her symptom onset date (or collection date of the earliest confirmatory test if onset date was unknown) between July 2, 2015, and August 3, 2015. *Legionella* subtyping, as described hereinafter, was used to refine the case definition.

Deaths from Legionnaires' disease were defined as (1) patients meeting the case definition whose death was attributed to Legionnaires' disease within 30 days of the diagnosis date, or (2) patients meeting the outbreak definition in which the Office of Chief Medical Examiner listed *Legionella pneumonia* as the immediate cause of death.

Analyses of Patient Characteristics

The patient demographic and clinical characteristics were summarized and adjusted odds ratios (aORs) and 95 percent confidence intervals (CIs) were calculated using multivariable logistic regression and the mid-P exact method to assess the relationship between fatality and comorbidities, smoking status, and number of days from onset to diagnosis. Odds ratios were adjusted for age and sex.

Environmental Monitoring

Cooling tower sampling in the outbreak zone was prioritized per the location of patients with Legionnaires' disease and the multi-focused cluster test. Although the city had no complete official registry of cooling towers at the time, cooling towers in the area were identified by examining city records of water credit and construction permit applications, in addition to publicly available satellite imagery. The sampled locations in cooling towers were thought to be most representative of the water aerosol generated. If the cooling tower basin was safe to access, a swab of biofilm was collected.

Methods. The New York State Department of Health Wadsworth Center and the New York City Public Health Laboratory tested cooling tower water samples for the presence of *Legionella* using PCR and culture methods. Use of PCR allowed for the rapid screening of samples to prioritize culture and cooling tower remediation. Samples in which *L. pneumophila* DNA was detected were processed and cultured at the Public Health Laboratory with standard microbiological methods. Isolates were identified as Lp1 through direct fluorescent antibody staining. Pulsed-field gel electrophoresis subtyping was performed at the Public Health Laboratory and Wadsworth Center with identical methods.

Epidemiologic Results

In total, 138 patients met the outbreak case definition of outbreak-associated Legionnaires' disease, and 128 (93 percent) were hospitalized. Illness onset peaked on July 26, 2015, and the last patient linked to the outbreak became ill on August 3, 2015. Sixteen (12 percent) patients died, five in their homes. A total of 108 patients (78 percent) resided in the outbreak zone. Of the remaining 30 patients, 16 resided in other Bronx ZIP codes, nine in other New York City counties, two in other New York State counties, and three in other states.

Several events led the investigation to one potential cooling tower source. On July 28, 2015, DOHMH received a physician inquiry about a cluster of respiratory illnesses among residents of a supportive housing residence for people with medical needs, including HIV infection, and the building identification number analysis identified two reports of Legionnaires' disease from this building. On July 29, 2015, the CDC notified DOHMH of a traveler who had been diagnosed with Legionnaires' disease and had spent part of the incubation period at a hotel in the South Bronx (Building A). Building A was located less than a block away from the supportive housing residence, and the cooling tower, which was not previously known to city agencies, was detected through satellite imagery. The multi-focused cluster test identified unusual case clustering of Legionnaires' disease cases around Building A, with a recurrence interval of 1.36 million years.

Environmental Results

The environmental investigation began on July 28, 2015. During the next three weeks, 55 cooling towers from 46 buildings in the outbreak zone were identified, inspected, and sampled. PCR results were available within 24 to 36 hours. Lp1 DNA was detected by qPCR in water samples from 21 cooling towers and successfully cultured from 14. An order to immediately remediate was issued to owners of cooling towers that tested positive for Lp1 by qPCR.

Whole-genome sequencing of the 14 Lp1 cooling tower isolates revealed the Building A strain to be indistinguishable from the 26 outbreak-associated clinical isolates. No strain from any other cooling tower matched to the Lp1 culture obtained from any patient during the investigation, as judged by whole-genome sequencing.

An order to disinfect all cooling towers within 14 days was issued to all NYC building owners on August 6, 2015. Tracking compliance with the citywide order presented its own difficulties, including the need to review more than 10,000 documents submitted to the city to demonstrate compliance.

Conclusions

A large outbreak of Legionnaires' disease resulted in severe illness and death in a NYC neighborhood. Epidemiologic, environmental, and laboratory investigations implicated a hotel cooling tower as the likely source of the outbreak. The outbreak response was expedited by a screening of water samples collected from cooling towers using a qPCR-based assay for Lp1 DNA followed by culture of PCR-positive cooling towers. Previous outbreak investigations relied on culture, which, if successful, can take several weeks to identify and subtype. Using qPCR allowed rapid screening, prioritization, and focusing of control efforts on potential outbreak sources.

Both host factors and environmental factors are likely to contribute to the increased number of cases of legionellosis since 2000. As discussed in Chapter 2, increasing numbers of persons are at higher risk of acquiring Legionnaires' disease because of aging of the population, increased use of immunosuppressant drugs, and higher prevalence of comorbid conditions, including diabetes and chronic obstructive pulmonary disease. There is a growing dependence on heating, ventilation, and cooling systems, as well as increased complexity of indoor plumbing systems in large buildings, which have a labyrinth of water lines and features ranging from hundreds of showerheads along lengthy hospital corridors to hot tubs and indoor decorative fountains. Changes in plumbing materials could play a factor. In addition, increased efforts to conserve water with attendant slower flow in plumbing systems likely enhances biofilm formation and therefore increases risk of *Legionella* growth in premise plumbing (see Chapter 4). Inadequate maintenance of public water supplies (e.g., water main breaks, corrosion of pipes) may increase risk for contamination of building water systems and other water devices or equipment. Contaminated environmental sources, from dental hygiene equipment to street cleaning machines, continue to be newly identified (Ricci et al., 2012; Schönning et al., 2017; Valero et al., 2017).

Changing environmental conditions are also facilitating human exposure to aerosolized water containing *Legionella*. Multiple hydrologic factors including humidity and rainfall may influence legionellosis risk, and climate change, including global warming, is likely contributing to the increase in cases (see Chapter 2).

Despite the increase in reported rates, most cases of legionellosis are not diagnosed, even among those who seek medical care, and there is little evidence that diagnostic testing has improved for legionellosis between 2007 and 2016. Diagnostic testing for pneumonia in the Unites States has been generally discouraged for many reasons. Reimbursement practices deter use of microbiologic diagnostic tests for pneumonia. Professional guidelines of the American Thoracic Society and the Infectious Disease Society of America have also discouraged routine testing of hospitalized patients for community-acquired pneumonia (Bartlett, 2011; Mandell et al., 2007). Although these guidelines are currently being updated, it is not expected that the guidelines' approach to legionellosis will change. At one academic medical center, adherence to these guidelines for testing of patients for *Legionella* would have resulted in an underestimate of the burden of Legionnaires' disease of at least 41 percent (Hollenbeck and Mermel, 2011). In this study, even with more robust testing than recommended by the guidelines, only 35 percent of patients discharged with a diagnosis of pneumonia had been tested.

Microbiologic analysis standards in most laboratories have declined. The belief that a deep respiratory secretion is needed for *Legionella* culture has discouraged testing, although this assumption is incorrect; sputum specimens that may be inadequate for culture of other pathogens may be sufficient for culture of *Legionella* (Bartlett, 2011; Ingram and Plouffe, 1994). In 2011, Bartlett reviewed reasons why testing has declined for diagnosis of community-acquired pneumonia. In particular, the Clinical

Laboratory Improvement Amendments regulations led to the demise of the "house staff laboratory" and the distancing of microbiological analysis from the site of care, which may delay diagnoses. Obviously, there are fewer options at most community and rural hospitals, many of which have only basic laboratories. Legionnaires' disease diagnostics, particularly use of culture, may have declined as a result of many of these factors. It is not known whether the use of PCR has had any impact on legionellosis diagnoses, although this may change as more molecular assays gain FDA approval.

There has been little, if any, federal research funding for applied research on legionellosis, which, in turn, may depress training on legionellosis in academic healthcare centers. As a result, academic healthcare centers in the Unites States have limited expertise on Legionnaires' disease. The National Institute of Allergy and Infectious Diseases has focused its *Legionella* funding on basic science related to *Legionella* and the pathogenesis of the organism (Heilman, 2015).

True Incidence of Legionellosis

It is difficult to determine from available data the true incidence of legionellosis in the United States, although reported cases are certainly an underestimate. Some studies have attempted to determine the incidence of Legionnaires' disease in hospitalized patients with pneumonia. A population-based study in two counties in Ohio in 1991 estimated 8,000 to 18,000 individuals were hospitalized with community-acquired Legionnaires' disease per year in the Unites States (Marston et al., 1997). From 2013 to 2015, 98 percent of patients with pneumonia in a Pittsburgh VHA hospital were tested for Legionnaires' disease with at least one diagnostic test, documenting that at least 1.7 percent of community-acquired pneumonia and 0.6 percent of healthcare-acquired pneumonia was caused by *Legionella* (Decker et al., 2016). The incidence of Legionnaires' disease among hospitalized patients was reported as 8/100,000 veterans, with an incidence of 6/100,000 for community-acquired Legionnaires' disease. More recently, Gamage et al. (2018) reported an incidence of Legionnaires' disease in the nationwide VHA system of 1.9/100,000 for the years 2014 to 2016. Since both VHA studies lacked data on veterans admitted to hospitals outside the VHA system, the incidence of pneumonia among veterans was underestimated. The CDC is currently working on better estimates of morbidity and mortality related to waterborne pathogens, including Legionnaires' disease, but these reports will not be available until late 2019.

To develop its own estimate of the incidence of Legionnaires' disease, the Committee relied on the estimate from the population-based Etiology of Pneumonia in the Community (EPIC) study of community-acquired pneumonia that required hospitalization (Jain et al., 2015). This CDC-led study is the more recent of only two such studies conducted in the United States that determined the incidence of Legionnaires' disease (the other being Marston et al., 1997). The EPIC study was conducted from 2010 to 2012 in Nashville, Tennessee, and Chicago, Illinois, and considered 2,488 patients. Using mainly UAT, Jain et al. estimated an incidence of community-acquired pneumonia caused by *L. pneumophila* of 4/100,000. Starting with this value, the Committee increased this rate to 4.44/100,000 after assuming a 90 percent sensitivity of the UAT for detection of *L. pneumophila* serogroup 1. This estimate is conservative; other have found that the UAT only detects of 80 percent of *L. pneumophila* serogroup 1 cases (Mercante and Winchell, 2015; Yzerman, 2001).

Another adjustment to the estimated incidence was made to account for the fact that the EPIC study was not designed to estimate Legionnaires' disease, and methods of enrollment and exclusion criteria (e.g., excluding immunosuppressed patients) as well as limited testing likely resulted in significant underestimates of the burden of community-acquired Legionnaires' disease. The Committee assumed that the enrollment and exclusion criteria removed at least 10 percent of actual cases, leading to a rate of 4.88/100,000 people. This adjustment is conservative given other, higher estimates of hospitalized

patients with community-acquired pneumonia. For example, Rameriz and colleagues (2017) studied adults hospitalized with pneumonia in Kentucky and reported rates of community-acquired pneumonia more than double those in the EPIC study and similar to rates found by Griffin et al. (2013), a study based on national Agency for Healthcare Research and Quality hospitalization data. Ramirez et al. (2017) attributed the higher rates in their study compared to those in EPIC to the stringent exclusion criteria used by EPIC.

Next, the Committee incorporated evidence (supported by Mercante and Winchell, 2015) that at least 20 percent of patients hospitalized with Legionnaires' disease have non-*L. pneumophila* serogroup1 disease, which was not captured in the EPIC study.[4] This consideration increased the rate to 6.17/100,000. The Committee then assumed that 10 percent of all legionellosis cases are healthcare-associated (see previous sections of this chapter), numbers which also would not have been captured in the EPIC study, leading to an adjusted rate of 6.85/100,000.

The EPIC study gathered and analyzed data from 2010 to 2012, such that the incidence cited in that study would reflect those years. According to Figure 3-3, there has been a doubling of the number of reported cases from 2011 to 2018, and this increase should be reflected in any current rate. There is little information available on the frequency of testing or whether diagnostic testing has improved (which could account for the observed doubling), has remained stable, or declined since 2011. The Committee assumed a range from as little as 50 percent of the doubling of reported cases being real (such that the other 50 percent is attributable to improved testing) to 100 percent of the doubling being real, which leads to a rate of 10.25 to 13.7/100,000. Although plausible, the Committee did not consider the possibility that diagnostic testing had decreased, a situation that would further increase its estimate of disease cases.

The U.S. Census Bureau on July 1, 2018, estimated there are 327.2 million people in the United States, of which 253.2 million are 18 years of age and older (children are excluded because there are limited data on estimates of Legionnaires' disease rates in children).[5] Thus, the Committee arrived at an estimate of 26,000 to 35,000 hospitalized cases of Legionnaires' disease per year.

The EPIC study considered only cases of community-acquired pneumonia that required hospitalization. To determine the incidence of outpatient Legionnaires' disease, the Committee consulted von Baum et al. (2008) who analyzed data from CAPNETZ, which is a medical competence network for community-acquired pneumonia funded by the German Ministry for Education and Research. von Baum et al. (2008) documented that the fraction of individuals with community-acquired pneumonia who were treated as outpatients was similar to that of persons with community-acquired pneumonia who were hospitalized. To be conservative, the Committee made a similar assumption, although there is evidence that, in the United States, the number of outpatients diagnosed with community-acquired pneumonia substantially exceeds the number of inpatients diagnosed with community acquired pneumonia.[6] Thus, **the Committee arrived at an estimate of 52,000 to 70,000 cases of Legionnaires' disease per year in the United States (or a rate of 20.5 to 27.4/100,000).** This estimate of the rate is approximately ten times higher than the reported rate for 2017 and is felt to be very conservative, as it considers only those cases of Legionnaires' disease for which treatment was sought (either inpatient or outpatient). It is a coarse analysis that does not reflect all of the uncertainties.

An analysis using different methods to estimate Legionnaires' disease in hospitalized patients with pneumonia provides further evidence that Legionnaires' disease may be substantially underdiagnosed in the United States. Cassell et al. (2019) reviewed hospitalization data for all non-federal hospitals in Connecticut from 2000 to 2014; using the International Classification of Diseases, they compiled time series for pneumonia and influenza, and estimated (with a mixed-effects model) the percentage of cases due to

[4] 31 of 32 EPIC cases were detected by UAT, with a single case detected by PCR. Cultures were not performed.

[5] See https://www.census.gov/quickfacts/fact/table/US/PST045218.

[6] See https://www.ahrq.gov/professionals/quality-patient-safety/hais/tools/ambulatory-care/cap-toolkit.html, accessed June 22, 2019.

Legionella, influenza, and respiratory syncytial virus. The annual incidence rate of Legionnaires' disease among hospitalized patients was predicted to be 11.7/100,000; this rate was also approximately ten times higher than the average reported rate during the 14-year study period. The estimates of the burden of Legionnaires' disease put forward by both the Committee and by Cassell et al. (2019) suggest that the U.S. rate of Legionnaires' disease may be far higher than that indicated by notifiable disease statistics.

ENVIRONMENTAL MONITORING

Monitoring of *Legionella* bacteria in water systems has been done for several reasons. Water sampling has often been undertaken to locate the source of the bacteria after an outbreak of Legionnaires' disease was documented or after cases began to accumulate. Routine monitoring is done to verify that a water management plan is working and to determine background levels of *Legionella*. For example, monitoring of cooling towers or hospitals, in the absence of cases of disease, has largely focused on whether or not to implement water treatment. Presence/absence approaches, where positive results initiate action, have frequently been used rather than quantitative measures. Assessment monitoring has often been done in conjunction with water treatment to determine treatment efficacy. Monitoring is also often carried out for research purposes, which is a valuable means of providing generalizable information to the scientific and practitioner communities about conditions in water systems that are conductive to *Legionella* growth and the means to control it. Table 3-1 provides a general overview of various methods currently available for environmental monitoring and how each may be applied toward these four goals. Of note, there is presently a great deal of variability in how the methods are actually applied to various systems and scenarios. This is likely because choosing the most appropriate methods, which systems and locations to target for testing and how often, and what medium to sample, are dependent on specific aspects of the water system and building being sampled. These are important considerations for a building's water management plan (discussed in Chapter 5). This section describes the individual methods and compares their strengths and weaknesses for various purposes. Finally, it summarizes what decades of data collection have revealed about *Legionella* presence and concentrations in various engineered, environmental niches.

Methods

Many of the methods used to analyze environmental samples for *Legionella* are the same as those discussed previously for clinical studies of Legionnaires' disease. Historically, culture-based methods have been applied as the standard method for monitoring and to obtain isolates for further characterization. However, new methods have been developed that shorten the delay inherent to culture methods and allow for more real-time information gathering.

The methods for environmental monitoring still do not fully account for *Legionella*'s complex ecology (see Chapter 2). For example, swabbing has been used as a sampling method because *Legionella* are known to be associated with biofilms that form in pipes and fixtures, yet quantitative data (e.g., area swabbed, method, other measures of total biomass obtained) have not been consistently reported. Few studies address the relationship of *Legionella* with amoeba and instead measure mostly planktonic bacteria. Recent knowledge of the ecology of *Legionella* spp. has been slow to impact the development of new methods, even in the research arena.

TABLE 3-1 Sampling for *Legionella* in Water Systems: Purpose, Methods, and Other Considerations

Purpose of Testing	Which Method(s)?		Which Water Systems?	Spatial/Temporal Considerations?	Which Medium/ Volume[a] to Sample?
Outbreak Investigation *Culture needed for comparison to patient isolates*	• qPCR/PCR- Rapidly identify suspect sites for further testing • Culture- Confirm viable *Legionella* • Serogroup, sequence typing, whole-genome sequencing- Compare to patient isolates		Suspect sources? Cooling towers, hot and cold taps, showerheads, hot tubs, decorative fountains, etc.	As soon as possible when an outbreak is suspected.	Water Numbers would be expected to be high in case of outbreak
Routine Monitoring *Select one, apply consistently*	• qPCR- Monitor baseline (viable + non-viable + VBNC) • Culture- Monitor baseline (viable and culturable)	• qPCR positives should be followed up by culture • Culture negatives- Be aware of VBNC • Either can be used to flag concerns and changes in system	Where there is patient risk, e.g., point-of-use devices in intensive care units, neonatal care units Where there is system vulnerability, e.g., stagnant zones, distal taps, substandard plumbing material	Continuous- Develop feasible plan and frequency (May be stipulated for some entities, locales, guidance, standards).	First draw water samples Biofilms are sampled routinely, but the value of these data over sampling of the water column unclear.
Mitigation Assessment *Select one or more, apply consistently*	• qPCR- Do numbers increase or decrease following mitigation? Note DNA from dead *Legionella* could still be detectable after disinfection. • Culture- Provides information on viability. • Amoebae co-culture- Evidence for VBNC forms? Changes in virulence?		The system subject to mitigation. Check upstream and downstream of target system and a comparable control.	Before and after mitigation, ideally long-term. Assess the overall effect or changes in baseline. Sample relevant inlets and outlets to point of mitigation.	Water Biofilm- Can assess if mitigation is reaching sources in biofilms
Research *Varies according to research question*	In addition to all of the methods above, consider: • Amplicon sequencing to address the responses of broader microbial community • Metagenomics—broader context of functional genes, viruses, other factors • Viability qPCR or flow cytometry—indicator of the viable fraction of *Legionella*		Water systems in place in the field. These are more real-world, but where there is a weaker understanding of factors at play. Simulated water systems. This allows for controlled variables and statistical replication, but less real-world significance.	Depends on research question. Longer-term studies are valuable but lacking. Water chemistry fluctuates with time. Three or more years may be required to achieve stable biofilm, which short-term studies overlook	Water Biofilm Aerosols- Need to understand transfer of *Legionella* from biofilms to respirable, infectious aerosols

[a] Volume to be determined based on application and desired detection limit. Larger volumes provide lower detection limits, but also may dilute the *Legionella* present in first-flush samples

Table 3-2 compares several methods in use for detection, isolation, characterization and quantification of *Legionella* from building water systems. The table includes whether the method (1) elicits a presence/absence or quantitative result; (2) allows the bacteria to be isolated; (3) can be used routinely; (4) identifies species, serogroups or genotypes; and (5) detects bacteria that are potentially viable, culturable, or those which are inactivated (killed). Each method has advantages and disadvantages. While culture methods have remained the gold standard, they may need to be adapted or supplemented with other methods to assist in developing risk estimates and informing outbreak investigations. Depending on the application, it is likely that combinations of methods will be used in the future.

Culture Methods

Culture methods capture cells that grow and produce colonies on solid agar, generating quantitative data in the form of colony forming units (CFU), or in some cases in liquid media. In many early studies using these methods, no quantification was undertaken because the goal was to isolate colonies and identify serogroups using antibodies. Thus, the methods initially focused on cultivation and isolation of the bacteria only. One major shortcoming that still exists today is the length of time it takes to culture *Legionella*, as results may not be available for eight or more days. This can result in precious time lost for outbreak investigation, but this delay is not typically problematic for routine monitoring.

By the late 1970s and early 1980s, media formulations were focused on growth of *L. pneumophila*, which led to the predominance of buffered charcoal yeast extract (BCYE) agar and the use of antibiotics as well as acid or heat pretreatment. The BCYE media used for culture tests is insufficient to recover all *Legionella* spp., although it does not exclusively detect *L. pneumophila* (Lee et al., 1993). Protocols that used filtration to sample larger volumes of water as well as swab samples became more prevalent (Cordes et al., 1981; Witherell et al., 1988). By 1990, improvements had been made, yet full assessment of a standard method was not forthcoming. There was concern regarding the standardization of the methods towards improved recovery and identification. After examining methods recommended by the VHA, CDC, and a group in Germany, Ta et al. (1995) made recommendations to enhance recovery of culturable species and identification of strains. Finally, in 1998 International Organization of Standardization (ISO) culture methods were updated and published (ISO, 1998). A variety of standardized and consensus-based methods are now available including *Standard Methods for the Examination of Water and Wastewater* (APHA, 2007); *Procedures for the Recovery of Legionella from the Environment* (CDC, 2005); and ISO methods ISO 11731-2 (100-ml membrane filtration) (ISO, 2004, 2017). Procedures were directed toward the isolation of culturable colonies, in part to facilitate comparison of environmental and clinical isolates during outbreak investigations.

A new, easier culture method specifically for *L. pneumophila* has been developed that uses a liquid-based most-probable-number (MPN) approach (Legiolert™/Quanti-Tray™, IDEXX). The comparative data from four studies (see Box 3-5) suggest that the method is equivalent to other methods but generally trends higher in concentration estimations, which could elicit more violations and trigger remediation more often. One limitation of the reported evaluations of the MPN method was the lack of confirmation tests on positive wells in the tray. None of the studies mentioned in Box 3-5 evaluated the positives with genetic confirmation, but tested only via culture. The method also does not differentiate among serogroups of *L. pneumophila* nor is its specificity for all 61 species of *Legionella* available, making further testing necessary if this information is needed. Another drawback of this MPN method is that cultures are not readily available for molecular discrimination assays. As new methods develop, there is a need for greater systematic study and reporting of information, including a full description of the types

TABLE 3-2 Comparison of Methods for Environmental *Legionella* Monitoring

Method	Potential for Quantification	Potential for Isolation	Level of Use*	Discerns Serogroups/ Sequence Types?	Form of Bacteria Measured	Pros	Cons
Culture Methods							
ISO	Yes	Yes	Routine	Yes	Culturable	Standardized Historical data	Time to results; may underestimate VBNC, other serogroups and species, risks
CDC	Yes	Yes	Routine	Yes	Culturable	Standardized Historical data	Time to results; may underestimate VBNC, other serogroups and species, risks
AHPA	Yes	Yes	Routine	Yes	Culturable	Standardized Historical data	Time to results; may underestimate VBNC, other serogroups and species, risks
*Molecular Methods^*							
PCR	No	No	Research, used with cultivation	No	Inactivated, VBNC+, Culturable	Can support sequencing	Need to process gels
qPCR	Yes	No	Research, potential for diagnostics and surveillance	No	Inactivated VBNC+ Culturable	Rapid results Greater sensitivity and specificity	Measures inactivated cells, less historical use
ddPCR	Yes	No	Research, potential for diagnostics and surveillance	No	Inactivated VBNC+ Cculturable	Rapid results Greater sensitivity and specificity	Measures inactivated cells; few studies using and comparing the method
Emerging Methods							
Next Generation Sequencing	No	No	Research	No	Inactivated VBNC+ Culturable	Provides info on how bacteria relate to microbial community	Takes special expertise, instrumentation. More cost and time to obtain results
Amoeba Co-culture	No	Yes	Research	Yes	Culturable	Improves isolation of difficult-to-culture strains	Adds at least 3 days to cultivation
Liquid-based MPN	Yes	Yes	Research, potential for routine use	No	Culturable	Simple set up, may be specific to Lp	8 days for results More difficult to confirm
EMA-PCR	Yes	No	Research	No	Viable	Can be used with molecular tools	Not proven to work with disinfection
PMA-PCR	Yes	No	Research	No	Viable	Can be used with molecular tools	Not proven to work with disinfection
Flow Cytometry	Yes	Yes	Research, potential for routine use	Yes	Inactivated VBNC+ Culturable	Simple set up, specific to Lp serogroups based on antibodies	Early commercial release, limited validation, higher detection limit

*Categories include Routine, Research, Potential for Routine, or Potential for Diagnostics and Surveillance; ^Molecular tools require special instruments, training, and expertise; VBNC+: Viable-but-Non-Culturable.

of samples compared, characterization of the genera and species eliciting false positives, and genetic characterization of the *Legionella* spp. and serogroups that are detected.

Although culture methods have been standardized, inter-laboratory precision and accuracy are still uncertain. In a methods comparison (Ta et al., 1995), filtration, use of BCYE agar, and acid buffer treatment gave the highest recoveries. One inter-laboratory study using seeded samples for proficiency testing examined how well various laboratories performed in detecting and quantifying *Legionella* (Lucas et al., 2011). Ten in-house protocols (which were not described in the paper) were used, based on American Society of Microbiology, ISO, or CDC methods. CDC and nine other laboratories including county, state, hospital, and private entities participated, with CDC as the reference laboratory. The key findings included the following:

- The detection limit of the methods and laboratories were similar; samples were negative 93.1 percent of the time with less than 10 CFU/mL and positive 85.3 percent of the time with samples with greater than 10 CFU/mL.
- Quantification errors averaged about 1 log and underestimated the expected concentrations. However, this conclusion was tenuous, as formal assessment of the quantification results were not clearly articulated in the publication.
- Statistics on accuracy and precision with only ten laboratories was similar to European studies. While the details were not provided, the study concluded that sampling protocol, treatment regimen, culture procedure, and laboratory experience did not significantly affect the accuracy of reported concentrations.

The advantages of culture include (1) its ability to compare with historical samples, (2) it is an accepted measure of viability, and (3) it can be used to isolate bacteria for epidemiologic investigations. The disadvantages are that final results are not available for eight to 14 days depending on the chosen laboratory, making rapid decisions impossible, and the cost and expertise needed to run the method limits its widespread use. Furthermore, the method cannot capture *Legionella* cells in the VBNC-like state, and it favors *L. pneumophila* and a few other *Legionella* spp., such that not all *Legionella* spp. associated with disease are identified (Lee et al., 1993). Approaches to recover the bacteria from the VBNC-like state have been reported (Oliver, 2005), including co-culture with *Acanthamoeba polyphaga* (Dusserre et al., 2008) as discussed below. Newer MPN methods may be easier to implement and, once fully vetted, could facilitate more widespread use by utilities, building owners, and public health laboratories.

Use of Amoeba

Amoeba co-culture for the recovery of legionellae from clinical and environmental samples was first described by Rowbottom (1980, 1983). While there are many bacterial pathogens that resist the digestive processes of predatory amoeba (so-called amoeba-resisting bacterial pathogens, Thomas et al., 2010), *L. pneumophila* is the most recognized in water systems (Corsaro et al., 2010; Tosetti et al., 2014). Amoeba of the genus *Acanthamoeba* are generally used for co-culture (Pagnier et al., 2008) because of the ease with which they are grown in cell culture, but different amoebal hosts and incubation temperatures may influence which specific *L. pneumophila* strains are recovered (Buse and Ashbolt, 2011). Use of amoeba from the local environment has also recovered *L. pneumophila* when other American Type Culture Collection (ATCC) *Acanthamoeba polyphaga* failed to recover any isolate (Dey et al., 2019).

BOX 3-5
Comparative Studies on Legiolert™

Four studies have evaluated *Legionella* occurrence and concentrations in side-by-side comparisons of Legiolert™, an MPN method in which the sample is distributed in a tray to generate a colorometric result after eight days of incubation, to other methods used more routinely. The first comparison (Satory et al., 2017) was against the ISO 11731-2 membrane filtration method with 290 paired samples. The second comparison (Petrisek and Hall, 2018) was against the standard culture method of APHA (2007) with 491 potable water samples and 846 nonpotable water samples. A third study (Rech et al., 2018) compared Legiolert™ to the CDC method (CDC, 2005) and examined 288 non-potable water samples. The fourth study (Spies et al., 2018) involved six laboratories comparing Legiolert™ (using 448 samples of 100 ml volumes) to ISO 11731-2 (100-ml membrane filtration) and ISO 11731 (1 ml direct plating). Table 3-5-1 provides the results. Confirmation is not a part of the MPN test as described, although cultured cells could be recovered for further testing/isolation.

TABLE 3-5-1 Comparative Studies on Legiolert™

Comparison Method	Sample Numbers	Sample Types	Results
ISO 11731-2 Membrane Filtration	290	Cold and hot taps showers circulation lines, boiler outlets	Overall, Legiolert™ provided a greater mean concentration. There were 3.3 percent false positives.
Standard Culture Methods (APHA, 2007)	491 846	Potable Water Non-potable water	There was no statistical difference between the methods; Legiolert™ < 0.5 percent and <0.9 percent false positivity rate for potable and non-potable samples, respectively. Did not mention a confirmation to *L. pneumophila* specifically**
CDC Method (CDC, 2005)	288	Non-potable water, mostly cooling towers	No differences were found between the methods. Non-pneumophila found in ten samples but only by the CDC method
ISO 11731-2 (100-mL membrane filtration) ISO 11731 (1 mL plating)	448	Cold and hot taps, showers, building circulation systems	For the 100-mL method, four of six laboratories had higher *Legionella* counts with the MPN method and the other two showed no difference. With the 1-mL method, five of six labs showed no difference. The specificity was found to be 97.9 percent.

** They confirmed 25 percent of the positive cells by recovering the liquid from the cells in the tray and re-isolating the bacteria on standard agar media.

Methods to recover amoebae from environmental samples are based on those developed over the past several decades. An environmental sample is applied to a lawn of viable *E. coli* prey on non-nutrient agar plates (e.g., 2% Neff's saline) and incubated at 25°C for up to two weeks, identifying any clearing zones with observable trophozoites moving away from the originally applied zone, and then re-streaking onto fresh plates (e.g., Amaro and Shuman, 2019; Lorenzo-Morales et al., 2005). The use of different prey and temperatures can recover a greater diversity of isolates, but is generally not undertaken.

To isolate legionellae using the amoeba co-culture method, an environmental water sample is incubated with amoeba obtained from a fresh, exponential culture using several dilutions to optimize the prey-to-host ratio, and then incubating the co-culture at 30°C for 12 hours. Co-cultures are observed by phase microscopy to identify trophozoites exhibiting lysis or growth of intracellular bacteria. Finally, the *Legionella* is isolated on BCYE agar.

Amoebae co-culture methods have not been standardized and have primarily been used in the research arena and in reference laboratories in Europe for water and clinical samples. This culture technique takes at least an additional three days, whereby the sample is first co-cultured, then the resulting amoebae-resisting bacteria are grown as usual on BCYE agar or are rapidly identified by qPCR/sequencing (e.g., Corsaro et al., 2009; Lienard et al., 2011). Advantages of co-culture are improved isolation and detection of viable microbes and recovery of isolates to compare to clinical isolates. Amoebae co-culture is also presumably biased toward *Legionella* that readily infect amoebae, thus serving as a proxy for virulence within human macrophages. The disadvantages of co-culture are lack of quantification, the time to obtain results, lack of standardization, and minimal information on its utility in routine monitoring.

PCR, qPCR, and dPCR

There has been significant growth in the use of molecular techniques either in combination or independently for detection and characterization of *Legionella* in environmental samples (Borges et al., 2012). PCR was first introduced in 1985 and initially provided presence/absence data. Today PCR kits that include appropriate standards and quality controls and instruments to run the test are widely available. PCR can be much less expensive than culturing *Legionella* and entails less time per sample, producing results in hours instead of days. Because it relies on DNA sequence recognition, PCR can provide very high specificity and confidence in detecting the intended target.

PCR works by cycling between high and low temperatures to separate and then anneal the DNA in a water sample. Specific, small pieces of DNA called primers direct the polymerase enzyme to copy a specific gene sequence. Finally, the genetic sequence of the DNA fragment that has been amplified is determined. The amount of target DNA produced each cycle increases exponentially, enabling easy visualization of the final PCR product by staining and verifying the correct molecular weight by size separation methods, such as electrophoresis. In practice, the water sample is initially filtered, the captured bacteria are removed from the filter and lysed, and their DNA is extracted for use as the template in the PCR amplification reaction. The method detects all cells in the sample, including culturable, inactivated, and VBNC-like cells, and potentially any DNA from dead organisms. PCR approaches are available for all species in the genus of *Legionella* (by analyzing the 16S or 23S rRNA gene), for *L. pneumophilia* (*mip* gene), and for *L. pneumophila* serogroup 1 (a region of the *wzm* gene, spanning nucleotides 99 to 392). Primer sets have also been published for *L. anisa, L. bozemanii, L. longbeachae* (Saint and Ho, 1999), and *L. micdadei* (Cross et al., 2016). The use of *L. pneumophila* serogroup 1-specific primers is relatively new, but appears to be gaining momentum since it was first introduced (Mérault et al., 2011).

More recently, quantitative PCR (qPCR) and droplet digital PCR (ddPCR) methods have been developed, which are a great improvement over traditional PCR in that they provide quantitative information. The quantitative units of qPCR and ddPCR are gene copies (GC) per unit volume (e.g., GC/L). qPCR works the same as traditional PCR, but it incorporates a dye or probe in the reaction and uses a

specialized instrument that can detect and quantify the signal as product is formed. Comparison of the exponential product amplification curves of samples to those generated by a standard curve of positive control DNA templates of known concentration allows quantification of gene copies per reaction. Units can then be converted to gene copies per volume of sample collected and subject to DNA extraction. ddPCR is a newer alternative to qPCR that provides rapid absolute quantification, without need for a standard curve, and is less sensitive to PCR inhibitors. Consequently, ddPCR can be applied to more than one genetic marker at a time, a procedure called multiplexing. The method works by dividing the sample into about 60,000 droplets wherein the PCR reaction occurs; the numbers of positive and negative droplets then provide a most probable number of the concentration.

Figure 3-5 provides the results from a seeded water sample using the primers and gene sequence for the genus *Legionella* (23S rRNA gene) and the *L. pneumophila*-specific *mip* gene.

Because qPCR and ddPCR capture all DNA, even from dead cells, more evaluation is needed before one could apply these methods during routine monitoring, particularly in environments containing high levels of disinfectants (e.g., cooling towers, hot tubs) where there is likely to be more DNA derived from dead cells. Culture and qPCR have been compared and contrasted for drinking water and cooling towers for detection of *L. pneumophila* and *L. pneumophila* serogroup 1 (Toplitsch et al., 2018). Twenty (20) drinking-water samples were examined, and the agreement was very good for *L. pneumophila* (90 percent positive by qPCR, 95 percent positive by culture, and 85 percent positive for both). In contrast, samples from cooling towers (n = 52) were scored as 60 percent positive using qPCR, 23 percent positive by culture methods, and 19 percent positive by both methods. For *L. pneumophila* serogroup 1, the agreement was poor for drinking water (10 percent, 5 percent, and 0 percent positive by qPCR, culture, or both, respectively), although slightly better for cooling towers (21 percent, 13 percent, and 4 percent positive by qPCR, culture, or both, respectively). When both tests were positive, generally qPCR reported 10- to 100-fold higher concentrations, although there was a positive correlation between the two tests. Another

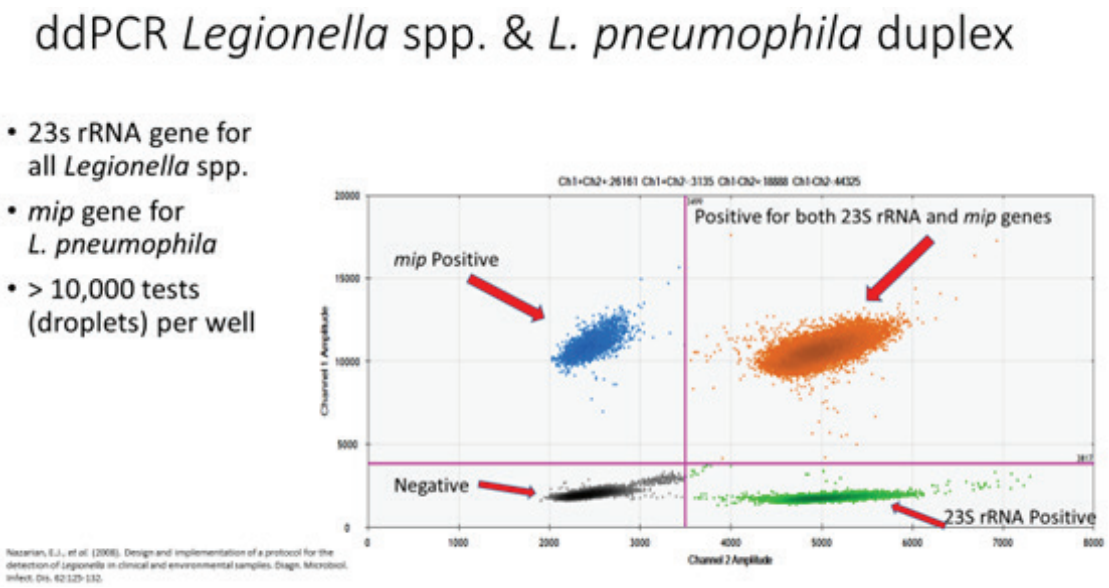

FIGURE 3-5 Results of a seeded water sample tested for two targets measured by ddPCR. The four quadrants show the number of droplets positive for the mip gene and the 23S gene (top right quadrant) or only the mip or 23S gene (left top and bottom right quadrants, respectively). The left bottom quadrant shows the number of droplets negative for both genes. Taken together, these results produce a most probable number for gene copies for both genes.
SOURCE: Courtesy of Joan Rose.

study similarly found that quantification of *L. pneumophila* by qPCR trends with that by culture in both hot water and cooling tower samples, but with consistently higher estimates (Yaradou et al., 2007). Lee et al. (2011) attempted to translate CFU/L into gene copies/L by comparing international results for both metrics from 232 cooling tower samples and 506 hot- and cold-water samples. There was a 2-log difference between qPCR (gene copies/L being higher) and culture (CFU/L) in cooling towers for *Legionella* species, but only a 0.71-log difference for *L. pneumophila*. For drinking water taps, there was a 1.05-log and 0.62-log difference between gene copies/L and CFU/L, respectively, for *Legionella* and *L. pneumophila*. PCR and culture-based tests can produce distinct results for several reasons. In addition to the capture of both VBNC-like and dead cells by PCR, variability in the distribution of the bacteria in any given water sample (e.g., one sample may have a clump of cells), differences in detection limits, efficiencies of the methods, and multiple gene or genome copies within a cell can result in different outcomes.

The advantages of qPCR and ddPCR include rapid results, the ability to design primers that have high specificity, and low cost, which allows for large numbers of samples to be tested. The disadvantages are that qPCR detects cells regardless of their viability. The use of PCR methods is becoming more widespread for clinical surveillance and outbreak detection and, if applied appropriately, could also be used for routine monitoring of water systems. Cooling towers are rarely monitored routinely by qPCR, in part because of the high concentrations of disinfectant and corresponding high levels of DNA from dead cells. However, even an increase in total *Legionella* DNA means that growth conditions are not being controlled somewhere in the system and is worthy of further investigation. When applied consistently, qPCR can be very useful for estimating baseline numbers of *Legionella*, even in disinfected systems, with increases and decreases indicative of growth and death in the system. Yaradou et al. (2007) noted good correspondence between qPCR and culture-based methods targeting *L. pneumophila* in cooling towers and suggested that qPCR could be adapted for more wide-scale cooling-tower monitoring in the future. It is not unprecedented to move from a culture-based method to qPCR, as was done for recreational waters (i.e., beaches) for *E.coli* and enterococci monitoring (Gonzalez and Noble, 2014). Now that there is an ISO method for qPCR detection of *Legionella* (ISO, 2019), it would be appropriate to compare the two methods (qPCR and a culture method) for a variety of buildings and water systems in order to help interpret qPCR-generated data. It is likely that greater application of qPCR will occur in the future given the speed with which qPCR can provide information.

Viability Analyses. To alleviate concerns that qPCR also detects non-viable bacteria, several methods have been developed that favor DNA (or RNA) detection and quantification of viable *Legionella*. One such method uses ethidium monoazide (EMA) or propodium monoazide (PMA) in combination with qPCR (Nocker et al., 2006; Nogva et al., 2003), referred to as viability qPCR. The first working principle is that on light exposure, both PMA and EMA bind to DNA and, as a result, this bound DNA can no longer be amplified by qPCR because the qPCR primers cannot bind to EMA/PMA-bound DNA (see Figure 3-6). Second, theoretically EMA and PMA cannot enter a cell when the cell membrane is intact, which is one of the viability parameters of a microbial cell (Hammes et al., 2011). As a result, free DNA and DNA from cells with a compromised membrane are bound with EMA or PMA, and that DNA will not be amplified during qPCR. In a similar way, cell integrity vital staining can be used in combination with flow cytometry.[7]

Viability qPCR has been used to quantify membrane-intact legionellae cells (e.g., Chen and Chang, 2010; Lizana et al., 2017). In general, these studies showed that when disinfected water samples were exposed to PMA or EMA, the gene copy numbers of *Legionella* calculated were between the number of *Legionella* colony forming units obtained by culture and the number of gene copies obtained with qPCR

[7] E.g., https://www.rqmicro.com/products/l-pneumophila-kit.

without PMA or EMA exposure. Accordingly, PMA or EMA seem to bind some of the *Legionella* DNA from membrane-intact cells that might still be viable after disinfection. However, serious precautions have been raised about the use of EMA and PMA to quantify viable *Legionella*, especially for environmental samples (Kirschner, 2016). These methods are not appropriate for studies involving a disinfectant whose mode of action does not affect membrane integrity, such as UV. Furthermore, there has been a lack of consistency among viability qPCR studies. For instance, the optimal EMA or PMA concentration for the viability assay reported in one study was shown to be cytotoxic to *Legionella* in another (Chang et al., 2010; Reyneke et al., 2017; Scaturro et al., 2016). In addition, the PMA method can overestimate viable *Legionella* cells (Scaturro et al., 2016). Moreover, Taylor et al. (2014) concluded that PMA is not an appropriate method for discriminating between live and dead *Legionella* cultivated under environmental conditions. Similar results have been obtained with EMA and PMA treatment of *Legionella* cells directly harvested from drinking water biofilms or cooling tower water, although the assay worked well with laboratory grown *Legionella* cells (Ditommaso et al., 2014; Wullings et al., 2016).

When compared to live/dead stain flow cytometry, viability qPCR for *L. pneumophila* overestimated membrane-intact cells when a large portion of the cells were membrane-compromised but underestimated membrane-intact cells when a large portion of the cells were membrane-intact. Thus, viability qPCR appears to be qualitative rather than quantitative. Furthermore, the performance of EMA and PMA treatment is much lower with shorter amplicon lengths (less than 200 base pairs or bp) than with larger amplicon lengths (greater than 400 bp) (Ditommaso et al., 2015; Wullings et al., 2016). Accordingly, larger qPCR gene targets of *Legionella* may be optimal. However, most companies providing molecular tools for qPCR recommend that amplicon lengths not exceed 200 bp for optimal qPCR. Kontchou and Nocker (2019) have recently optimized the PMA assay for *L. pneumophila*, which includes a longer amplicon (633 bp), higher incubation temperature, and addition of EDTA and deoxycholate. They determined

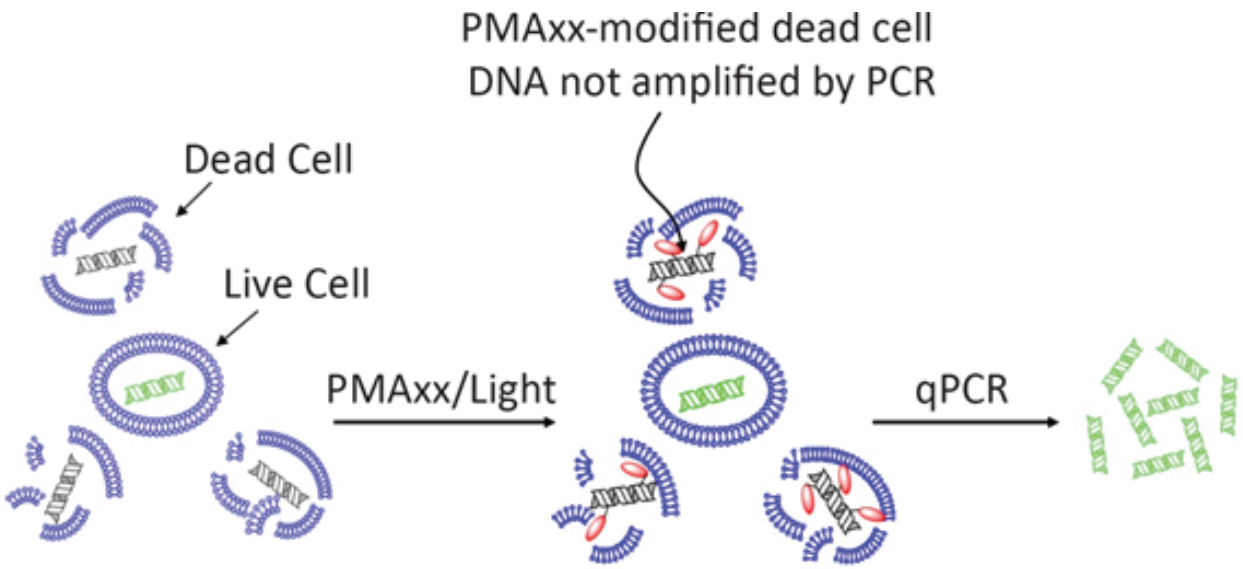

FIGURE 3-6 Principles of live/dead quantification with PMA and qPCR.
SOURCE: https://biotium.com/product/viability-pcr-starter-kits. Image by Biotium® Inc.

that the membrane-intact *L. pneumophila* cell numbers obtained with PMA-qPCR were in agreement with membrane-intact cell numbers obtained with flow cytometry, demonstrating potential for this optimized assay, with the caveat that *L. pneumophila* strains were cultivated under optimal conditions. Overall it can be concluded that, although PMA or EMA treatment in combination with qPCR might have merit to distinguish between membrane-intact and membrane-compromised *Legionella*, additional studies on the reliability of the method, standardization of the method, and its application to environmental samples need to be performed before qPCR assays can be applied routinely to detect viable *Legionella*.

Another promising molecular method that distinguishes between viable and nonviable *Legionella* detects precursor RNA, which is only produced by viable cells on exposure to fresh nutrients (Cangelosi and Meschke, 2014). To detect *L. pneumophila* by assaying for precursor RNA, samples are exposed to fresh nutrients for three hours, RNA is extracted, and then RNA from the precursor region of the 16S rRNA gene of *L. pneumophila* is specifically amplified with reverse transcriptase (such that the method is called RT-qPCR) (Boss et al., 2018). In one study, *L. pneumophila* in drinking water samples taken from public sport facilities was analyzed by RT-qPCR, cultivation, and qPCR. For 86 percent of the samples, the results with RT-qPCR and cultivation were consistent. In 7 percent of the samples the culture method was positive but RT-qPCR was negative, whereas in the other 7 percent of the samples RT-qPCR was positive but culture was negative. In addition, 17 percent of the samples that were negative with RT-qPCR were positive with qPCR, indicating the presence of DNA from dead *L. pneumophila*. Others have also used RT-qPCR to detect RNA of specific genes (including virulence genes) of *L. pneumophila* after exposure to synthetic grey water (Buse et al., 2015) or copper (Lu et al., 2013). The specific analysis of virulence genes in these assays might not only provide information on viable *L. pneumophila* cells but also on their virulence potential. Although RT-qPCR seems promising, additional studies are needed in which RT-qPCR results are compared with cultivation, qPCR, and viability qPCR for detection and quantification of *Legionella* in different environmental samples.

Next Generation DNA Sequencing

A handful of studies have used next-generation DNA sequencing approaches to examine *Legionella* or other relevant members of the microbial community in drinking water systems. Amplicon sequencing is one application that is applied to amplified PCR products obtained from DNA extracted from mixed microbial communities. Most often amplicon sequencing uses universal primers for bacterial 16S rRNA genes to profile which organisms are in a particular drinking water or biofilm sample. Organisms are identified based on the similarity of the 16S rRNA gene sequence to entries in online databases, and the term operational taxonomic unit (OTU) defines the bacteria identified. Because at best the resolution is at the genus level, the presence of pathogens cannot be ascertained.

Nevertheless, amplicon sequencing has proved to be a powerful tool to reveal the surprising diversity of microorganisms inhabiting drinking water (Pinto et al., 2012) as well as estimate their relative abundance. In one laboratory study of domestic hot water, qPCR and amplicon-sequencing-based methods estimated *Legionella* spp. to be around 3 percent of the total community (Ji et al., 2018). Next-generation DNA sequencing can be applied directly to the DNA extract, without first PCR-amplifying a gene of interest, an approach referred to as shotgun metagenomic sequencing. The advantage of shotgun metagenomic sequencing is its potential to sequence *all* genes in a sample, including markers of function (e.g., nitrification, iron oxidation, virulence), and thus provide much richer functional information and taxonomic resolution (Gomez-Alvarez et al., 2012). However, currently metagenomic sequencing is very costly; consequently, researchers tend to employ less thorough sequencing, which results in false nega-

tives because of high detection limits and lack of coverage. Both amplicon sequencing and metagenomic sequencing also provide rich information about non-*Legionella* species in water systems and could potentially provide new insight into the role of microbial ecology in *Legionella* propagation (Dai et al., 2018). However, for potential application to *Legionella* monitoring, these tools are still in their infancy (Borthong et al., 2018). In the future, next-generation sequencing of both environmental and human isolates could potentially provide insight into the relationship between environmentally abundant *Legionella* and disease and perhaps help to identify previously unidentified clusters of disease.

The third application of next-generation sequencing is whole genome sequencing of individual *Legionella* isolates (Reuter et al., 2013). Whole genome sequencing makes possible high-resolution phylogenetic comparisons of isolates associated with outbreaks, and it can also be adapted to determine the sequence type (Raphael et al., 2016). Raphael et al. (2019) have used whole genome sequencing on cultures of clinical specimens to reveal a highly diverse population of strains causing legionellosis in Arizona.

Sampling Strategy

A *Legionella* monitoring plan for water systems should include (1) the purpose of the monitoring, (2) what medium to sample, (3) the method to be used, and (4) where and when to sample. As discussed in Chapter 5, the precise sampling strategy should be developed and adapted to the system of interest as part of a comprehensive water management plan (see the example in Box 3-6). Monitoring for *Legionella* in building water systems can have many purposes including to investigate outbreaks, to support remediation or mitigation, to demonstrate compliance with a guideline or regulation, as part of diagnostic surveys, and for research (see Table 3-1). Once the purpose is determined, the methods should be linked to the desired information. The priority may be confirmation or quantification, determining viability, or distinguishing serogroups or sequence type. For example, culture and viability are of interest when disinfection is being used for remediation. For compliance monitoring, the methods are usually prescribed. Surveys generally attempt to use standardized methods to facilitate comparison. Nonetheless, newer methods such as qPCR have great potential to quantitatively examine more samples at a lower cost and much more rapidly. Legiolert™ may enable greater ease in sampling at a lower cost than current culture methods, although the time to receive results remains a week.

First, the water system to be sampled must be identified, such as cooling towers, residences, public buildings like hotels, resorts, hospitals, drinking water, and wastewater. In particular, points thought to be most vulnerable to *Legionella* growth and where potential for human exposure is high should be prioritized. For example, within buildings, premise plumbing monitoring should include distal sites that have potential both for *Legionella* growth and human exposure; these include showers and taps, decorative fountains, and storage tanks. Although *Legionella* growth is less likely in the hotter water of recirculation lines and water heaters, sampling these locations is also important for confirmation and to provide a baseline.

The various media that can be targeted for sampling include the bulk water, biofilm, or the aerosols generated. Most sampling strategies and methods have focused on the bulk water because it is easy to collect, various volumes can be readily targeted, and it can be concentrated via filtration. In addition, first-flush samples are thought to capture water that has been stagnating (thus more likely allowing for bacterial growth), potentially better representing what has sloughed or diffused off of the biofilm. (It should be noted that most studies lack any quantitative assessment of stagnation. For example, a study of 807 drinking water samples from nine buildings found occurrences to be significantly correlated with stagnation, but this was described only qualitatively as "low withdrawals" [Völker et al., 2016)]).

Legionella bacteria are known to associate with biofilms and their amoeba. However, swab samples have had limited value in decision making for remediation of premise plumbing. Swabs are not analyzed routinely because it is impossible to collect a representative biofilm sample from the miles of premise plumbing in a building, there is no standard method available, and there is no consistent way to report the concentrations found. Developing better methods for sampling premise plumbing biofilms is clearly a research need.

Because aerosol sampling is much more complicated than sampling the bulk water and still under development, aerosols are generally not included in a sampling strategy. Nonetheless, aerosols can be collected as they are generated using various types of impingers or impactors. A research program to understand the difference between measured *Legionella* concentrations in bulk water and in aerosols would be useful (Prussin et al., 2017).

The detection methods applied should include more than one technology (likely a culture method and a molecular method, e.g., qPCR) and be quantitative. Laboratories will continue to use culture but may use more than one medium; this may be unnecessary if, for example, qPCR or dPCR was used first to examine more rapidly the concentrations of specific species or *L. pneumophila* serogroup 1. The detection limit should be carefully documented, addressing both the volume collected and concentrated. More experience is needed where both types of results (culture and molecular methods) are available, thus providing knowledge on their comparability. Sivaganesan et al. (2019) has compared qPCR methods to culture for fecal indicator bacteria on beaches over many years during the swim season. These data are now being analyzed in several states to address the comparable level of gene copies per 100 ml that would lead to beach closure on the same day rather than waiting 24 hours to obtain culture data. A similar approach could be used for *Legionella*.

The frequency of environmental sampling for *Legionella* is highly variable and ranges from once per week to once per year, depending on many factors such as the size and use of the building. Box 3-6 describes the *Legionella* sampling strategy applicable to large buildings with complex premise plumbing systems such as hospitals, while Box 3-7 describes the sampling strategy for cooling towers; both boxes prescribe sampling frequencies. In general, however, the numbers of samples taken and how often they are collected have been based on resources and logistics rather than on an understanding of the ecological niche of the bacteria. Temporal studies with recommendations on how often to monitor and over what time frame have yet to be undertaken. Nor has there been a clear statistical assessment of the frequency of sampling needed to capture *Legionella* growth, blooming, and sloughing events. To evaluate temporal changes such as seasonality, several years of monitoring would be needed.

More widespread and improved national laboratory certification is needed for current approaches and for new methods, which includes standardized protocols, quantitative assessment, training and proficiency testing. The Environmental *Legionella* Isolation Techniques Evaluation (ELITE) Program has oversight from the CDC, but since November 2016, the Wisconsin State Laboratory of Hygiene has managed the production and distribution of testing samples as well as analysis of laboratory results. Twice per year, participating laboratories receive cultures for verification tests.[8] The program issues certificates to laboratories that successfully isolate legionellae from simulated environmental samples by culture, but it is not a laboratory certification process. New York State certifies laboratories[9] as do the Quebec and Alberta provincial governments in Canada.

[8] See https://wwwn.cdc.gov/elite/Public/FAQ.aspx.
[9] See https://www.wadsworth.org/regulatory/elap.

BOX 3-6
***Legionella* Sampling Strategy in Large Buildings**

Large buildings, including hospitals, which house vulnerable populations, tend to formalize their management of *Legionella* in building water management plans (see Chapter 5 for more description of these plans). Although not universal, these plans often require some form of *Legionella* sampling to gauge the effectiveness of the building's water treatment system and to determine if the treatment needs to be modified to maintain plan effectiveness. Sampling strategies are unique to any given building, and both the water management plan and the sampling strategy are subject to change as surrounding and contributing environmental conditions change.

A frequently used guidance for developing a water management plan is the American Society of Heating, Refrigeration and Air-Conditioning Engineers (ASHRAE) standard 188 (ASHRAE, 2015). This standard does not provide a sampling strategy nor address biological testing for *Legionella*, but it does reference a companion document ASHRAE 12-2000 (ASHRAE, 2000) which states that "culturing for *Legionella* may be appropriate if carried out for a specific purpose, such as verifying the effectiveness of a water treatment protocol." The CDC toolkit (CDC, 2017a), which aims to make ASHRAE 188 more practical, also makes reference to environmental testing for *Legionella* to validate the effectiveness of control measures.

Once water temperature, disinfectant residual, and distal point flushing programs have been considered to aid in identifying sampling locations and potential *Legionella* growth risks, *Legionella* sampling should be the basis for validating any water management plan, regardless of building size, configuration, or even building population composition, which are risk factors secondary to plan development. Initial samples will define the extent or even if *Legionella* is present and the extent to which the plan should be developed. In very general terms, the initial sampling would include bulk water samples from the water source entering the building, from storage tanks if used, and from both hot and cold water distal sites at multiple points in the building. Initial sample draws should be evaluated by competent third party entities rather than contractors or vendors who are responsible for mitigation modalities. Samples should be tested by culture (e.g., ISO, 2017) or by qPCR (e.g., ISO, 2019). When sampling, it is highly recommended to record specific sample location, temperature, disinfectant residual, pH, and plumbing zone flushing and usage. These additional data points will minimize resampling time and define the conditions contributing to any given water management issue. Both culture-based and qPCR-based monitoring must be taken into context and compared to a baseline, not interpreted in isolation.

Following initial testing, water management plan development will move forward based on the test results and other associated risk factors unique to the building. At this point, *Legionella* sampling will evaluate changes to the plan and any required mitigation to maintain water safety for water uses within the building.

For large buildings, the building manager will need to identify the potential locations where *Legionella* may be present and propagate, based on the number of potable water systems and the number of distribution components. Examples are as follows:

- Potable sources: Some building configurations have multiple water mains. A sample should be taken from each source.
- Potable tanks: If used, potable tanks should be tested. Water tanks will extend the age of water.
- Potable zones: Larger buildings, particularly high-rise buildings, may have multiple building zones as a result of building height and pressures. A sample should be taken from each zone.
- Distribution risers: Samples should be drawn from enough risers to provide a good evaluation of all risers. If sampling and testing is done frequently enough, i.e., monthly, a random selection of risers would be possible. Selection should always include risers in which water use is minimal.
- Horizontal distribution: Samples should be taken from enough of the horizontal distribution to be representative of the entire length. At a minimum, the end point of the horizontal distribution should be sampled to determine whether mitigation is reaching the farthest point of the system.

- Potable hot-water: All system points listed above should be tested for both hot- and cold-water systems. *Legionella* is more likely to exist and propagate in hot-water systems where temperatures range from 29.4°C to 40.6°C (85°F to 105°F). More hot-water points should be sampled than cold.
- Potable hot-water heat exchangers: Where used, they should be evaluated and sampled.
- Potable hot-water return piping: Where used, it should be sampled, as conditions are often suitable for *Legionella* growth.

Once the locations that will provide a good indication of system performance are identified, the interval for sampling can be determined. In cases where initial testing indicated there was no presence of *Legionella* anywhere in a facility, and the building use composition indicated no risk of exposure to building occupants, sampling may be once every six months or even once per year. The sampling interval is also driven by the building's risk tolerance. A hospital with a large immunocompromised patient population and zero tolerance for *Legionella* may opt for more frequent sampling. In either case, the sampling strategy is dictated by risk and water management plan parameters.

BOX 3-7
***Legionella* Sampling Strategy for Cooling Towers**

For routine maintenance of cooling towers, a *Legionella* sampling program is key in ensuring that the operation and maintenance activities, as well as the water treatment, are effective. In the event of a Legionnaires' disease outbreak associated with a cooling tower, a well-planned sampling strategy can help to isolate specific components of the cooling tower system that are responsible. This sampling strategy should identify or rule out suspected sources and their transmission pathways.

A thorough visual assessment of the cooling tower should be conducted prior to any sampling, to determine the condition of the various components of the cooling tower and their potential to amplify and transmit bacteria. Table 1.3 in HSG274 Part 1 (HSE, 2013) denotes the various parts of a cooling tower and Figures 1.5 and 1.6 are photographs of the cooling tower fill conditions (see www.hse.gov.uk/pubns/priced/hsg274part1.pdf). Operation and maintenance records, as well as water treatment records and any past sampling data, should also be examined for gaps or unusual results as well as follow-up actions and validation results.

Once the equipment and its components have been visually inspected, a sampling plan should be devised to take into account any potential problems. Sampling locations for cooling towers should include the locations listed below, either in routine sampling or during an outbreak. However, these locations will vary depending on the cooling tower's components. The CDC (2015) sampling procedure for outbreaks of disease, shown in Table 3-7-1 below, indicates the number and type of samples, and the targeted process for each location.

Table 3-7-1 *Legionella* Sampling for Cooling Towers SOURCE: CDC (2015)

Cooling towers[a]			
Make-up water (water added to replace water loss because of evaporation, drift, or leakage)	1	1L bulk water	Direct
Collection basin (an area below the tower where cooled water is collected and directed to the sump)	2	1L bulk water and a biofilm swab at the water line	Direct
Sump (a depressed chamber contiguous to the basin, where water flows to facilitate pump suction; may also be used as collection point for silt and sludge)	2	1L bulk water and a biofilm swab at the water line	Direct
Storage tank or reservoir in the system	1	1L bulk water	Direct
Drift eliminators or other surfaces that remain moist	1	1 biofilm swab	Direct
Heat sources (e.g., chillers)	1	1L bulk water	Direct

Various documents such as AIHA (2015) and PWGSC (2013) provide additional guidance regarding the type of samples to be collected, the sampling locations, the frequency, and the proper handling and analytical methods for both routine and outbreak monitoring of cooling towers. The recommended frequency for routine *Legionella* monitoring may vary from weekly (PWGSC, 2013) to quarterly (HSG274, 2013) depending on the outcomes of the visual inspection, past issues, and targeted outcomes. Other physical parameters to monitor include temperature, pH, residual (free) chlorine, other disinfectant levels, and water flow rates.

When preparing a routine sampling strategy for cooling towers, it should be noted that:

1. Cooling towers tend to operate fully only during the summer months, although some will be operated sporadically throughout the winter depending on cooling needs.
2. Cooling towers do not continuously circulate water even when they are in operation, which provides conditions ideal for *Legionella* growth.
3. Cooling towers are frequently not accessible for inspection or sampling.
4. The plumbing configuration for a group of cooling towers can be very confusing. Therefore, it is necessary to properly trace the piping for each tower to pinpoint the sampling locations that will reflect the conditions for any given cooling tower and its associated equipment.
5. Because cooling towers are essential to the operation of modern buildings, it is difficult to take a cooling tower offline to allow for inspection, sampling, disinfection, or repair. A heat transfer plan should be part of the sampling strategy.
6. Surveillance monitoring is completed on a regularly scheduled basis. However, it is recommended to vary the testing time for comparison purposes (e.g., after a long weekend, mornings, afternoons). The same applies to locations within a system when possible, for example, by sampling different heights of fill of the cooling tower.
7. Personal protection equipment including eye and respiratory protection and anti-slip footwear should always be used when working around cooling towers.

Occurrence of *Legionella* in Water Systems

Much of the emphasis for environmental sampling of *Legionella* has been to understand its occurrence and (in some cases) concentrations in different locations. Sampling has focused on sites where aerosols that might contain the bacteria are formed, including cooling towers, showers, hot tubs, fountains, and buildings with vulnerable populations (e.g., hospitals). Over the years, better methods and lower detection limits have increased the percentage of samples that test positive for *Legionella*, yet concentrations have remained variable. Despite this variability, a general picture regarding the occurrence of the genus, its various species, and serogroups is emerging.

The sections below present occurrence and (when available) concentration data on cooling towers, residences, hotels and resorts, recreational venues, hospitals, cruise ships, and drinking water and wastewater treatment plants. The data were generated using either culture methods that quantify colony forming units (CFU) and include cells that grow and produce colonies on solid agar, or qPCR for which the data are referred to as gene copies (GC) and that include live, VBNC-like, and dead cells with intact DNA. Data presented below represent both outbreak investigations as well as routine sampling.

Cooling Towers

Legionella data from cooling towers were collected from general surveys conducted in the absence of outbreaks as well as from outbreak investigations. One of the first studies to collect environmental data on *Legionella* in cooling towers was conducted in 1983 (Howland and Pope, 1983). Nine cooling towers were routinely sampled over an 18-month period (162 samples). The culture methods used only identified presumptive *L. pneumophila,* which was found in all samples and all systems (100 percent positive). The levels were noted to be higher in systems that were used seasonally (i.e., shutdown in the winter and drained); however, the data were not presented in detail.

In 1983, a 12-city study took place to investigate *Legionella* in potable water and cooling towers in Canada (Tobin et al., 1986). Calgary, Edmonton, Fredericton, Halifax, Mississauga, Montreal, Ottawa, Poplar River, Quebec City, Regina, Winnipeg, and Vancouver were part of the survey. Sampling occurred from July to September, using a 1- to 2-liter sample that was filtered and plated on BYCE agar. Of the cooling towers that were specifically examined, 28.9 percent of the samples were positive. *Legionella* concentrations in cooling towers were a maximum of 3.3×10^4 CFU/L with a geometric mean of 4×10^3 CFU/L. Almost all isolates were *L. pneumophila* species including serogroups 1, 3, 4, and 6. One isolate was *L. dumoffii*.

A 2016 study collected 196 cooling tower samples across various regions of the United States (Llewellyn et al., 2017). In this study, 62 percent were positive by qPCR for *Legionella* spp., 32 percent were positive for *L. pneumophila,* and 20 percent were positive for *L. pneumophila* serogroup 1. The authors cultured only PCR-positive samples and found that 47 percent were positive for *Legionella* spp., 32 percent were positive for *L. pneumophila,* and 24 percent were positive for *L. pneumophila* serogroup 1. No concentrations were reported and no geographic differences were found.

A study of cooling towers in Singapore was one of the few conducted in a tropical environment (Lam et al., 2011). Over an eight-year period (2000–2008), 18,164 samples were analyzed by culture methods and 15.6 percent were positive for *Legionella*. However, a greater prevalence of positivity was found in the first three years, ranging from 48 to 68 percent, which then dropped to between 12 and 15 percent from 2004 to 2008. Although it was speculated that this decline was because of the switch to chloramines, the drop occurred prior to implementing the change in disinfectants (which was in 2005). Again, concentrations were not reported.

Investigations into 255 industrial cooling towers in China revealed a positivity rate of 37 percent using culture techniques (Li et al., 2015). 121 isolates were characterized and all were *L. pneumophila*, mostly serogroup 1 (56.2 percent), although serogroups 6, 5, 8, 3, and 9 (at 20.7, 9.9, 6.6, 5.0, and 1.6 percent, respectively) were also identified. Concentrations between 100 CFU/L and 88,000 CFU/L were reported, with an average of 9,100 CFU/L.

Widespread monitoring of cooling towers in New York City was undertaken during an outbreak of Legionnaires' disease from November 2014 to January 2015. This included power plant cooling towers, in which 29 of 30 samples were positive by PCR (although primers or genes examined were not mentioned), as well as shopping mall cooling towers, in which eight of ten were positive by PCR for *L. pneumophila*. Those that were positive were cultured, and 90 percent (27/30) and 12 percent (1/8) from the power plants and shopping mall cooling towers, respectively, were positive for *L. pneumophila* serogroup1 using serology (Benowitz et al., 2018). Concentrations were not reported in these studies. The methods used are poorly described, with no indication of the detection limit for the sampling.

Walser et al. (2014) summarized 19 outbreaks associated with cooling towers from around the world, nine of which had environmental sampling data. Interestingly, the *Legionella* concentrations were greater than 5×10^5 CFU/L and as high as 1×10^8 CFU/L with an average 1.4×10^7 CFU/L, with the exception of one outbreak from Norway (2×10^3 CFU/L). These concentrations are above the average found in the Chinese studies of 9.1×10^3 CFU/L. Attack rates were not calculated because it was unknown how

many people were exposed to the cooling towers. The concentrations were not related to the number of cases or cases/day, although there was a positive relationship between duration of the outbreak and concentrations.

Residences and Public Buildings

Surveillance of *Legionella* in residential premise plumbing taps and showers has been undertaken in many parts of the world because of the concerns associated with sporadic cases of Legionnaires' disease in a community that cannot be linked to hospitals, hotels, or cooling towers. Some studies have linked an individual with Legionnaires' disease to a source within their residence, such as Chen et al. (2002). *L. pneumophila* serogroup 6 was isolated from both the patient and his home potable water system as confirmed by pulsed-field gel electrophoresis (a method used to fingerprint DNA from bacteria). Other studies implemented over the past 30 years have tried to broadly survey environmental data from residences in China, Germany, Italy, Spain, the United Kingdom (UK), and the United States. In some cases, there was an attempt to examine levels of *Legionella* in taps in homes or areas of a city where Legionnaires' disease cases had occurred (Stout et al., 1992).

The data from 11 studies are shown in Figure 3-7. Taken together, these data show that the percentages of samples positive for *L. pneumophila* (using culture methods for *Legionella* followed by colony confirmation test specific to *L. pneumophila*) ranged from 5 percent to as high as 33 percent. When culture methods for *Legionella* (without colony confirmation testing) were used, positives ranged from 8 percent to 23 percent. As expected, qPCR reported higher numbers of positive samples for *Legionella* spp. (28 percent to 100 percent) but not notably higher for *L. pneumophila* (3 percent to 64 percent). Average concentrations for *L. pneumophila* reported in the various studies were 1.1×10^3 CFU/L (in the UK), $3\text{-}5 \times 10^3$ CFU/L (Spain) and 1×10^4 to 6×10^5 CFU /L (Pittsburgh). Using qPCR approaches, concentrations were reported at 4.0×10^3 GC/L for *L. pneumophila* (UK) and 10^4 GC/L (China). For other *Legionella* species, the concentrations were 1.2×10^4 GC/L (UK) and 7.7×10^4 to 8.4×10^6 GC/L (China). Levels were found at 10^5 GC/L for *Legionella* spp. in rain barrels (where no *L. pneumophila* was detected).

Insights are provided by the studies in Figure 3-7. Stout et al. (1992) found *L. pneumophila* was associated with lower water temperatures in water heaters (at or below 41°C), with no prevalence in any particular kind of tap. While many suggest warm-water taps should be sampled, the data suggest that all taps can be positive. In China, *L. pneumophila* was more frequently found in public buildings than in residential buildings, perhaps because of higher water age (Liet al., 2018). In public buildings in China, negative correlations were noted between *Legionella* numbers and total chlorine residuals and between total 16S rRNA gene copy numbers and total chlorine in both the first draw and post flushing (Li et al., 2018). Storage appeared to increase *Legionella* numbers, which were slightly higher in underground systems (average $1.95 \times 10^6 \pm 2.49\ 10^6$ GC /L) compared to rooftop storage ($7.8 \times 10^5 \pm 1.40 \times 10^6$ GC/L, $P < 0.05$).

A German study (Dilger et al., 2018) involved 76,200 samples taken from 13,397 warm-water systems. Ninety-four (94) percent were private homes, with the rest being schools, town halls, sports facilities, hotels, hospitals, and retirement homes. While the average *Legionella* concentration was not reported, 14 percent had less than 10^3 CFU/L (reported per 100 mL in the paper, i.e., 100 CFU/100mL) and 0.19 percent had 10^4 to 10^5CFU/L (which according to German standards is a level at which showering would be restricted). 20.7 percent of samples were positive for *Legionella* spp., of which *L. pneumophila* was the prominent species (83.9 percent) followed by *L. anisa*, and 12 other species. The differences in abundance of the various species detected was partly explained by temperatures, as *L. pneumophila* was present at all temperatures from 10°C to 60°C, while *L. anisa* was more abundant at low temperatures and other species were limited to narrower temperature ranges.

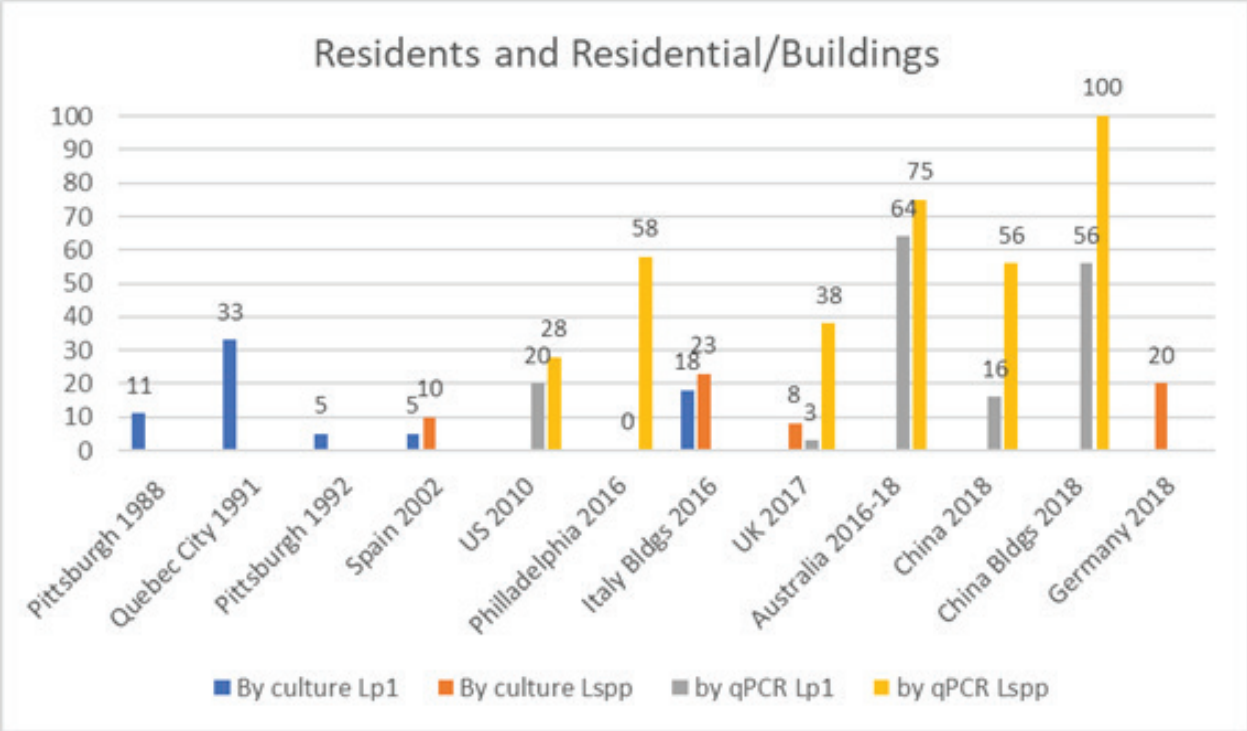

FIGURE 3-7 Percentage of samples positive for *L. pneumophila* and other species using culture and PCR from publication dates of 1988 to 2018 in large surveillance studies of households. SOURCES: Pittsburgh 1988 (Lee et al., 1988); Quebec City (Alary and Joly, 1991); Pittsburgh 1992 (Stout et al., 1992); Spain (Codony et al., 2002); U.S. 2010 (Donohue et al., 2014); Philadelphia (Hamilton et al., 2018b); Italy (Totaro et al., 2017); UK (Collins et al., 2017); both China columns (Li et al., 2018); Germany (Dilger et al., 2018).

Higher *Acanthamoeba* concentrations in taps fed by tanks compared to those fed by mains were reported in the studies in Hong Kong, Korea, and the UK (Boost et al., 2008; Jeong and Yu, 2005; Seal et al., 1992). *L. pneumophila*, *Acanthamoeba*, and *V. vermiformis* were also detected in tank and tap water in the Chinese study (Li et al., 2018).

Donohue et al. (2014) surveyed 68 public and private cold-water taps from 2009 to 2010. Low concentrations of *L. pneumophila* serogroup 1 were found, between 40 and 620 GC/L, in around 50 percent of the positive samples; yet on occasion, a high level was found up to 10^5 GC/L, creating an average of 1.97 x10^3 GC/L with a median of 62 GC/L. This study found that 47 percent of sampled drinking fountains were contaminated with *L. pneumophila* serogroup 1, with 18 percent of the fountains (3/17) consistently positive.

The prevalence of *Legionella* in hot and cold water was investigated in 141 homes equipped with various types of domestic water heaters (38 percent gas, 38 percent electric, 18 percent oil, and 7 percent solar) in four regions of France (Wallet et al., 2016). Samples by culture exceeded 1,000 CFU/L in 5 percent of hot water and 5.6 percent of cold water from mixing valves and taps. Results using solid phase cytometry for *Legionella* were strikingly higher, with a prevalence of 41 percent in hot water, 52 percent for cold water, and 53 percent for mixed water.

Verhoef et al. (2004) showed that *Legionella* was present more often in homes that had not been inhabited for ten days than those that had been occupied. Although the results were not significant, the study suggested that some Legionnaires' disease attributed to temporary accommodation sites (e.g., hotels) might be due to domestic exposure.

A study in Australia examined the occurrence and concentrations of *Legionella* in home showers using qPCR (Hayes-Phillips et al., 2019). *Legionella* spp. and *L. pneumophila* were positive in 74.6 percent (50/68) and 64.2 percent (43/68) of the showers, respectively. The researchers also demonstrated that qPCR had the potential to demonstrate increased growth potential of the bacteria and exposures at temperatures between 40°C and 60°C.

Hotels and Resorts

Legionella is frequently found in hotels and resorts. Papadakis et al. (2018) collected 518 samples from 119 hotels in Crete and assayed them by culture; of these, 36 percent (n = 43/119) of the hotels and 13 percent of the samples (n = 67/518) tested positive. The majority of positive samples were from swimming pool showers (see Figure 3-8). Like many studies, few samples (n = 5) tested positive for *L. pneumophila* serogroup 1. Figure 3-9 and Table 3-3 show the distribution of species, serogroups, and concentrations, respectively. The concentrations of *L. pneumophila* serogroup 1 ranged from 3.5×10^2 to 1.15×10^3 CFU/L. This study is similar to many surveys where a range of isolates is found, with concentrations similar to those previously reported.

In Flint, Michigan, 16 samples from hotels and schools were collected from 2015 (during the Legionnaires' disease outbreak) to 2016 (after the outbreak). No *L. pneumophila* was detected, but about 50 percent of the samples were positive for *Legionella* spp. by qPCR at 2.3×10^3 GC/L (Rhoads et al., 2017).

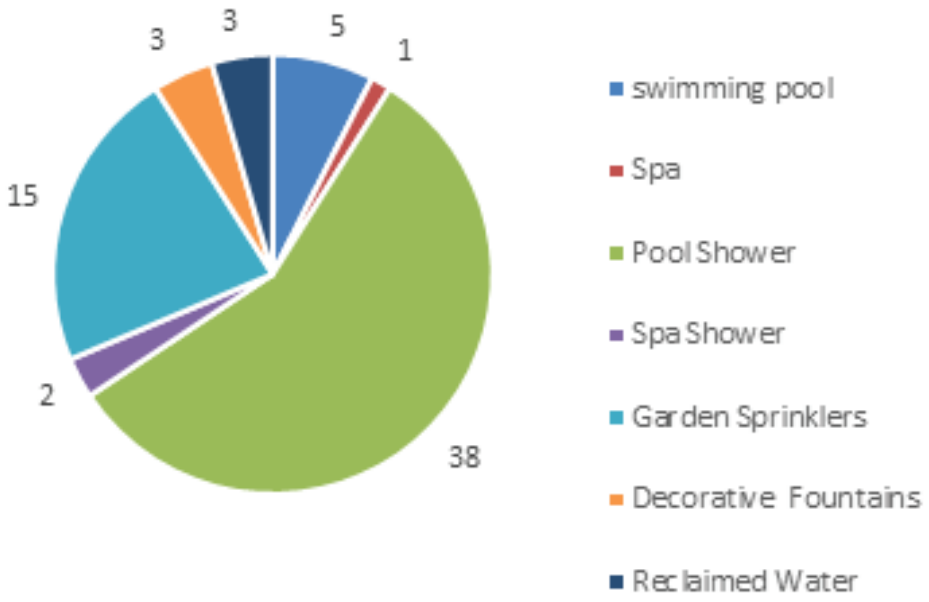

FIGURE 3-8 Note no detection in hotel showers, Jacuzzis, or soil; however, only 2, 15, and 2 samples were collected from these locations, respectively. SOURCE: Papadakis et al. (2018).

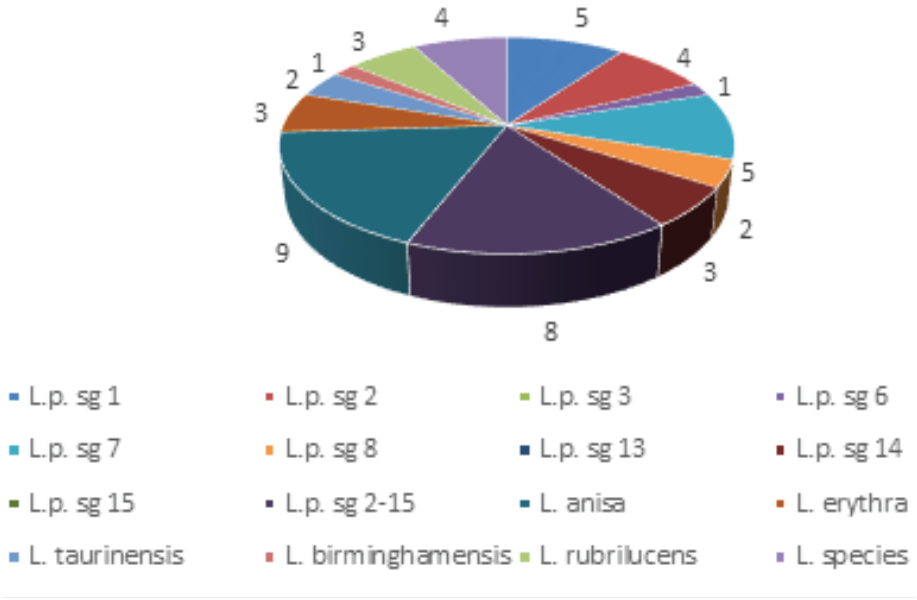

FIGURE 3-9 Distribution of *Legionella* species and serogroups detected in hotel swimming pool showers by culture. SOURCE: Papadakis et al. (2018).

TABLE 3-3 Concentration Ranges of *Legionella* Species and Serogroups Detected in Hotel Swimming Pool Showers by Culture

Species/Serogroup	# of Positive Samples	Pool Shower Low CFU/L	Pool Shower High CG
L.p. sg 1	5	350	1150
L.p. sg 2	4	100	2050
L.p. sg 3	0		
L.p. sg 6	1		150
L.p. sg 7	5	200	3350
L.p. sg 8	1		50
L.p. sg 13	0		
L.p. sg 14	3	150	100,000
L.p. sg 15	0		
L.p. sg 2-15	8	50	100,000
L. anisa	9	250	300,000
L. erythra	3	400	13,000
L. taurinensis	2	650	8,250
L. birminghamensis	1		50
L. rubrilucens	3	50	6,500
L. species	4	50	1,000

SOURCE: Papadakis et al. (2018)

In a study of 51 hotels in Greece and Corfu that had been linked to travel-associated Legionnaires' disease (via epidemiological methods although no outbreaks were identified), Kyritsi et al. (2018) reported that 74.5 percent of the hotels were colonized with *Legionella* spp. The study took place between October 2011 and December 2012, and hygienic inspections and physiochemical data were also collected. Samples were primarily collected from showers (n = 496), with a few others from swimming pools (n = 36), taps (n = 8), coolers (n = 2), boilers (n = 3), cold-water tanks (n = 3), hot tubs (n = 4), cooling towers (n = 3) and one fountain, for a total of 556 samples. For each sample, 500 mL were filtered and assayed by culture methods with a detection limit of 100 CFU/L. In hot- and cold-water taps, *L. pneumophila* was found in 76.8 percent of the samples (with *L. pneumophila* serogroup 1 and *L. pneumophila* serogroups 2-15 at positive rates of 35.8 percent and 41.4 percent, respectively). Non-*pneumophila Legionella* was detected in 10.9 percent of the samples. Detection was greater in hot water (41 percent positive) and hot tubs (75 percent) compared to cold-water samples (21.4 percent). Those systems with copper piping had samples that were 12.1 percent positive versus 30.4 percent positive in systems without copper. Free chlorine levels of greater than 0.375 mg/L were negatively associated with *Legionella*. The following parameters were positively associated with *Legionella* in the cold-water systems (pH > 7.45, heterotrophic bacteria $\geq 2.5 \times 10^4$ CFU/mL, conductivity $\geq$ 1,775 uS/cm (at 25°C), hardness $\geq$ 321 mg $CaCO_3$/L, and calcium concentrations $\geq$ 150 mg $CaCO_3$/L) (Kyritsi et al., 2018). The regulations in Greece set a limit of 10^3 CFU/L for *Legionella.* Some of the hotels in this study that were deemed unsatisfactory using parameters such as hygiene and chlorine were also above this limit for *Legionella*.

Recreational Venues. Recreational sources such as hot tubs and hot-spring baths have long been associated with outbreaks of Legionnaires' disease and Pontiac fever, primarily caused by *L. pneumophila* serogroup 1. Table 3-4 shows the concentration data collected from recreational waters by Leoni et al. (2018) during outbreak investigations that included environmental monitoring using culture techniques. The *Legionella* concentrations were generally greater than 10^5 CFU/L in these outbreaks, with little association among cases, attack rates, and concentrations. Pontiac fever outbreaks showed much higher attack rates than Legionnaires' disease.

TABLE 3-4 Attack Rates, Case Numbers, and Legionella Concentrations of Selected Outbreaks of Recreational Waters

Venue	Attack Rate (%)	Cases	Concentrations (CFU/L)
Pontiac Fever			
indoor whirlpool	38	13	1.00E+06
hotel whirl spa	66-72	45	9.00E+04
resort spa	86	6	100
Legionnaires' Disease			
public bathhouse	0.13	23	8.80E+05
public bathhouse	0.2	34	8.42E+04
hot-spring bath	1.5	295	1.60E+06
public bathhouse	0.13	9	1.30E+06
public whirlpool spa	?	3	1.50E+05

SOURCE: Leoni et al. (2018).

Hospitals

There is great concern about *Legionella* infections in hospitals because of their susceptible populations. As mentioned in Box 3-6, in many large hospitals *Legionella* monitoring has been undertaken to confirm that water treatment is suppressing bacterial growth in the premise plumbing. The goal for most hospitals is to detect no *Legionella*. Monitoring is undertaken to provide assurance to patients and managers of the building that controls are working. Culture methods are used most frequently, and any positive results tend to instigate investigation and remediation.

Stout et al. (2007) examined *Legionella* culture data from 20 hospitals in 14 U.S. states between 2000 to 2002 (see Table 3-5). As few as ten and as many as 80 samples were collected per hospital. *Legionella* (specifically *L. pneumophila* serogroup 1, *L. pneumophila* serogroups 2-14, and *L. anisa*) was detected in 70 percent of the hospitals. These investigators characterized "high level colonization" as when 30 percent or more of the distal outlets were positive for *L. pneumophila*. A total of 668 samples were collected and 21.4 percent were positive for *L. pneumophila* serogroup 1, 9.4 percent for *L. pneumophila* serogroups 2-14, and 9.9 percent for *L. anisa.* At hospitals that were positive, the percentages ranged from 5 to 83 percent, 5 to 67 percent, and 4 to 28 percent for *L. pneumophila* serogroup 1, *L. pneumophila* serogroups 2-14, and *L. anisa,* respectively. Eleven (11) hospitals had *L. pneumophila* serogroup 1 but only four of these had known cases of Legionnaires' disease.

TABLE 3-5 *Legionella* Detection in Premise Plumbing of 20 Hospitals

Hospital Location	Cases of Legionellosis Identified	>30% of distal water outlets positive for *L. pneumophila*	*L. pneumophila* sg 1 %+ (#+/total)	*L. pneumophila* sg 2-14 %+ (#+/total)	*L. anisa* %+ (#+/total)
CA	Yes	Yes	47 (7/15)	0 (0/15)	13 (2/15)
PA	Yes	Yes	30 (12/40)	25 (10/40)	0 (0/40)
NY	Yes	Yes	36 (8/22)	0 (0/22)	0 (0/22)
IA	Yes	Yes	35 (19/55)	0 (0/55))	0 (0/55)
NE	No	Yes	83 (58/70)	0 (0/70)	24 (17/70)
OH	No	No	25 (11/44)	0 (0/44)	0 (0/44)
AZ	No	No	20 (10/49)	12 (6/49)	16 (8/49)
MI	No	No	5 (2/44)	14 (6/44)	7 (3/44)
FL	No	No	17 (2/12)	0 (0/12)	8 (1/2)
WV	No	No	12 (7/58)	0 (0/58)	12 (7/58)
CA	No	No	7 (3/42)	0 (0/42)	0 (0/42)
OH	No	No	0 (0/57)	67 (38/57)	28 (16/57)
TN	No	No	0 (0/28)	7 (2/28)	4 (1/28)
MA	No	No	0 (0/20)	5 (1/20)	0 (0/20)
KY	No	No	0 (0/10)	0 (0/10)	0 (0/10)
MI	No	No	0 (0/44)	0 (0/44)	0 (0/44)
DE	No	No	0 (0/23)	0 (0/23)	9 (2/23)
NY	No	No	0 (0/12)	0 (0/12)	0 (0/12)
NY	No	No	0 (0/13)	0 (0/13)	0 (0/13)
MI	No	No	0 (0/10)	0 (0/10)	0 (0/10)

SOURCE: Stout et al. (2007)

Two hospitals in Flint, Michigan, were tested after an outbreak of Legionnaires' disease in 2014 and 2015. The prevalence and concentrations of *Legionella* from October 2015 and March 2016 were measured using qPCR (see Table 3-6 and Rhoads et al., 2017). These two time points corresponded to before and after the Flint drinking water was switched from the Flint River back to Lake Huron; October 2015 was also identified as near the end of the outbreak. The percent positives ranged from 3 to 74 percent for *L. pneumophila* and from 29 to 94 percent for *Legionella* spp. Concentrations in the positive samples were similar (10^3 GC/L), regardless of the percent positive. Nonetheless, both percent positives and concentrations were considerably higher in October 2015 compared to March 2016.

Although dozens of hospitals are monitoring for *Legionella*, long-term monitoring data are not readily available. Box 3-8 describes the *Legionella* monitoring program and its results, as well as the engineering approaches used, in one hospital after a decade of testing the water in the hospital's premise plumbing. This extensive database suggests that non-detects can be achieved and that improvements in water treatment of hospital plumbing systems assist in achieving this outcome.

Monitoring has also been used to prove that remediation efforts in hospitals are successful after an outbreak. A nosocomial outbreak of Legionnaires' disease in 2013 in Australia was followed by extensive cleaning of the water system using heat, flushing, and chlorination (Bartley et al., 2016). The environmental monitoring used culture methods, which attempted to match the clinical isolates to water isolates from the patients' rooms (showers and taps were cultured). Overall 18 percent of the water samples were positive for *L. pneumophila* serogroup 1 ranging from 6.3 percent to 71.4 percent positive in one of the wings of the hospital. The premise plumbing was treated with 60°C water for ten minutes, yet positive samples were still detected (5/89, 5.6 percent). Disinfection was then carried out by flushing the system with a chlorinated alkaline detergent (pH = 10.0) and then superchlorinating with 10 mg/L free chlorine. Three cycles of treatment were needed to rid the hospital of *Legionella*.

TABLE 3-6 Percentage of Samples Positive and Average Concentrations for *Legionella* spp. and *L. pneumophila* at Hospitals in Flint, Michigan, October 2015 and March 2016, by qPCR

Locations	Total # of samples	Lp # +	% Positive	Average Concentration GC/L	L spp. # +	% Positive	Average Concentration GC/L
October 2015							
Total	98	51	52	3,000	80	82	3,300
Hospital A	46	34	74	3,000	43	94	3,400
Hospital B	52	17	33	3,000	36	69	3,100
March 2016							
Total	44	1	2	Below quantification	16	36	2,300
Hospital A	35	1	3	Below quantification	10	29	2,500
Healthcare facility	9	0	0	Below quantification	6	67	1,900
Grand total	142	52	36.5		96	67.7	

SOURCE: Rhoads et al. (2017).
Note: GC=gene copy detected by qPCR.

BOX 3-8
Hospital Monitoring: Reviewing an 11-year Data Set

A hospital on the east side of NYC has maintained records of *Legionella* testing of its potable water for more than a decade. From 2007 to 2017 there were only three positive cultures. Interestingly, the positive cultures were all found in bulk water samples of distal sites, while swab samples from the same sites gathered at the same time tested negative. This analysis describes the testing methodology and system configuration, and it reviews potential conclusions that may be drawn from the results.

History. The hospital includes a high-rise tower with less than 500 registered inpatient beds. The hospital sees approximately 23,500 inpatients per year in this facility. The patient population is primarily immunocompromised and is highly susceptible to waterborne pathogens, including *Legionella*. In 1999, the hospital experienced what potentially was the first nosocomial case of *Legionella*. The patient was diagnosed with *Legionella jordanis* and *Legionella bozemanii* serogroup 2. Both *Legionella* types were also detected in environmental samples of potable water in the hospital.

Potable Water System. The primary water source to the hospital is the NYC water supply. Hospital floors at basement, ground, first, second and third levels are supplied from street pressure; all other floors (4 to 21) are supplied from two gravity roof tanks. Inpatient beds are on floors 4 to 19. Two wooden water tanks are located on the roof, each with a total capacity of 10,000 gallons, of which 4,750 gallons are held as fire reserve and 5,000 gallons are available for domestic use.

The hospital's water heaters are the instantaneous type with minimal storage capacity. Temperature is set at 60°C (140°F) and mixed locally at faucets and shower bodies. Circulating pumps on the hot-water returns operate in a continuous mode.

Inpatient bathrooms, sinks and showers, nurse server sinks and all other potable distal sites from the fourth to the 19th floor are fed from 18 pairs of hot and cold risers. Water distribution begins in the ceiling of the 19th floor and ends in the ceiling of the third floor. Hot-water returns with balancing valves are at the base of each hot-water riser and return back to the heaters on the 20th floor.

Secondary Water Treatment. Following the first diagnosed Legionnaires' disease patient in 1999, the hospital installed secondary water treatment to prevent *Legionella* growth and propagation in the building plumbing in March 2000. Research and discussions with the hospital's infection control group indicated that long-term mitigation should primarily address the potable hot-water system. To ensure effective treatment levels were maintained, quarterly water testing for *Legionella* was performed after secondary treatment was installed; these longitudinal records provided the basis and the data for this review.

Water Testing Protocol. During the analysis period, potable water testing was performed quarterly. The bulk of the samples were taken twice at each distal site, once by swab and once a first draw of bulk water. All samples were drawn and sent to a third-party lab, overnight delivery, in lab-provided containers. The swabs were taken from inside the faucet/shower with the screen/head removed.

Water Testing Dataset. The review of sampling data began in March 2007 and continued through December 2017. Both water column and swab samples were collected quarterly from approximately 40 to 46 locations that were either showers or faucets. Showers represented 85 percent of the samples collected (1,445 total samples, half were swabs) and faucets represented the other 15 percent (253 samples, half were swabs). Of the 1,698 samples collected over the 11 years, only three were positive. One sample from a shower was positive for *L. pneumophila* serogroup 1 (140 CFU/mL) and the other two positive samples were *Legionella anisa* (8 CFU/mL and 10 CFU/mL from a faucet and shower, respectively). The faucet sample positive for *L. anisa* was taken at a sink in a newly renovated ICU prior to occupancy. It should be noted that while

the shower water tested positive, the swab samples taken at the same time from the same location tested negative. All tests were performed by culture; the detection limits were 10 CFU/swab sample and 1 CFU/mL for the water sample. It is unclear if seasonality was involved, although the positives were found in spring and fall (March and September). Engineering data such as disinfectant residual, pH, temperature, and estimated water age were not measured or recorded at the time of sampling.

Conclusions. Ten years of quarterly testing were performed from 2007 to 2017. During this period, 1,698 tests were performed, resulting in three positive cultures. The three positives were all obtained from the bulk water samples while the corresponding duplicate swab samples were negative. Over the 11-year period of testing, and after the implementation of secondary water treatment, the level of positivity was reduced to two-tenths of one percent, substantially below the 28 percent positivity rate at system implementation measured in the year 2000.

Cruise Ships and Ferries

Goutziana et al. (2008) studied *Legionella* on cruise ships and ferries in Greece. No *Legionella* was found in the ten cruise ships' water systems. However, 14 of the 21 ferries were positive when 276 samples of hot and cold water were analyzed, and remediation commenced. There was greater contamination in the ferries' hot-water systems, with 38, 34, 19, 15, and 7 percent of the samples positive for *Legionella* spp., *L. pneumophila*, *L. pneumophila* serogroup 1, *L. pneumophila* serogroups 2-14, and *L. pneumophila* serogroup 1 concurrent with other serogroups, respectively. In cold water, 18, 15, 11, 4, and 2 percent of the samples were positive for *Legionella* spp., *L. pneumophila*, *L. pneumophila* serogroup 1, *L. pneumophila* serogroups 2-14, and *L. pneumophila* serogroup 1 concurrent with other serogroups, respectively. In another similar study, 12 cruise ships were found to be negative for *Legionella*, while 28 ferries were sampled and found to be positive 81 percent of the time (Mouchtouri and Rudge, 2015).

Drinking Water and Wastewater

Many fewer monitoring studies have focused on drinking water or wastewater systems compared to the other categories, with most studies undertaken as investigative special surveillance studies. A national study found *Legionella* spp. in 12 of 18 samples (67 percent positive by qPCR) from the sediments of drinking water storage tanks of ten states (i.e., Alabama, Arizona, California, Illinois, New Jersey, North Carolina, Ohio, Pennsylvania, Tennessee) at average concentrations of 5.2 x 10^3 cell equivalents(CE)/gram of wet weight of sediment (Lu et al., 2015). *L. pneumophila* was found in 33 percent of the samples and *L. pneumophila* serogroup 1 was found in 28 percent. (To facilitate comparison with other studies, dry weight rather than wet weight should have been recorded.) Developing consensus on methods and data reporting is needed for these types of investigations in order to begin to build national databases and to understand the role of drinking water in seeding of premise plumbing.

Drinking water and reclaimed water were examined for *Legionella* species by Garner et al. (2018) using qPCR (see Table 3-7). Prevalence was higher in reclaimed water compared to potable water (89 percent versus 55 percent), and concentrations of gene copies were 10- to 100-fold higher in reclaimed water. There was no quantification of *L. pneumophila*, although it was annotated in samples using metagenomic approaches.

TABLE 3-7 *Legionella* spp. by qPCR in Potable and Reclaimed Waters and Biofilms

Sample	% Positive (n=)	Gene Copy/L
Potable water POE	67 (15)	5.6×10^5
Potable water POU	56 (102)	4.7×10^5
Reclaimed water POE	91 (22)	3.8×10^7
Reclaimed water POU	87 (96)	9.6×10^7
Swabs from potable water POU	52 (60)	1.9×10^5
Swabs from reclaimed water POU	92 (51)	5.6×10^6

Note: averages in the final column were determined from positive samples only. POE refers to the point of entry to the distribution system while POU refers to the point of use from the distribution system. SOURCE: Garner et al. (2018).

The best known example of a drinking water source playing a major role in an outbreak of Legionnaires' disease occurred in Flint, Michigan, in 2014–2015. The outbreak coincided with a change in the source and treatment of drinking water for the City of Flint. In the absence of proper chemical corrosion control, this change in source water led to drastic increases in iron levels in the water and also risked disrupting biofilms coating the surfaces of pipes, releasing *Legionella* into the potable water supply of many buildings. Box 3-9 discusses this case in greater detail.

Wastewater treatment plants have been identified as sources for Legionnaires' disease or Pontiac fever in different countries. In 2013, a large outbreak of legionellosis (159 cases) occurred in Warstein, Germany. The source for the outbreak was a cooling tower that received river water into which a biological wastewater treatment plant discharged (Maisa et al., 2015). The effluent of this wastewater treatment plant contained high numbers of *L. pneumophila* (approximately 10^7 CFU/L), and genotyping showed identical patterns in patient strains and strains from the wastewater treatment plant (Maisa et al., 2015). Investigations at the treatment plant showed that the aerobically pre-treated wastewater contained high numbers of cultivable legionellae (10^8 to 10^{10} CFU/L) (Noguiera et al., 2016), demonstrating that legionellae were capable of multiplying in this treatment process.

BOX 3-9
2014- 2015 Legionnaires' Disease Outbreaks in Flint, Michigan

Flint is an industrial city in Genesee County, Michigan, whose economy boomed in the 1960s. Subsequent changes in the auto industry decreased factory jobs, with unemployment peaking around 17 percent in 2009 and reaching about 5 percent in 2018 (Bureau of Labor Statistics). By 2018 the Flint population had decreased by about 50 percent. Since 1954, the municipal water source was Flint River water treated at the Flint Water plant. In 1967, the city began purchasing water from Detroit Water and Sewerage Department (DWSD), which treats Lake Huron water at the Fort Gratiot plant. To reduce costs, in April 2014, the city switched back to Flint River water treated at the Flint Water plant. However, corrosion control measures were inadequate. Within a few weeks of the switch, residents complained of not only red and smelly water, but also skin rashes, respiratory irritation, and gastrointestinal problems. By the end of 2014, the Genesee County Health Department also recognized an increase in legionellosis cases, a pattern that repeated in the summer of 2015. In October 2015, the municipal water was switched back to DWSD, with appropriate corrosion control. A massive flushing program was also in place through spring of 2016. At that point, 79 Legionnaires' disease cases and 12 deaths had been reported. By the following summer, legionellosis cases had declined to historic baseline rates (Rhoads et al., 2017; Zahran et al., 2018). Table 3-9-1 gives a timeline of the Flint water crisis events.

TABLE 3-9-1 Timeline of the 2014 - 2015 Flint Water Crisis

April 2014	Michigan Department of Environmental Quality (DEQ) approves source water switch and a Flint River changeover ceremony is held. On April 25th, the community begins receiving treated Flint River water.
May 2014	Complaints begin of poor water quality (smell, taste, discoloration).
June 2014	**6 cases of legionellosis occur.**
August 2014	Flint water tests positive for E. coli. Two boil water advisories are issued.
September 2014	**32 total cases of legionellosis have occurred.**
November 2014	City increases hydrant flushing to address red water concerns.
December 2014	City receives official violation notice from DEQ for violations of the Safe Drinking Water Act (SDWA) for total trihalomethanes (TTHMs).
End of 2014	**42 total cases of Legionellosis have occurred in 2014.**
February 2015	High levels of lead are found at a residence (up to 397 ppb).
May 2015	**3 cases of legionellosis occur.**
June 2015	**9 cases of Legionellosis occur.**
June 2015	City receives second violation notice from DEQ for violations of the SDWA for TTHMs.
July 2015	Flint installs a granular activated carbon filter to control TTHMs by removing organic matter.
September 2015	**44 total cases of Legionellosis have occurred in 2015.**
October 2015	Flint switches back to DWSD-treated water from Lake Huron.
October 2015	Governor Snyder appoints the Flint Water Advisory Task Force to investigate.
December 2015	For corrosion control, Flint increases phosphate concentration from 1 to 2.5 mg/L.
January 2016	Federal emergency declared by President Obama.
March 2016	Flint Water Advisory Task Force Report issued.
May 2016	Massive "Flush for Flint" campaign to ensure corrosion control is delivered throughout.
Summer 2016	Legionnaires' disease cases return to pre-2014 levels.

SOURCE: Adapted from Masten et al., 2016.

Epidemiology. During the period that Flint residents received treated Flint River water, their risk of Legionnaires' disease was elevated 6.3-fold (Figure 3-9-1A). That the switch in source and treatment of the municipal water system accounted for this increased disease risk was supported by additional independent epidemiological analyses. When purported hospital-associated cases were disregarded, the risk remained elevated by a factor of 5.7. After boil-water advisories were announced, the odds of Legionnaires' disease cases among Flint residents declined, most likely due to reduced water use among Flint residents after the boil-water advisory (Zahran et al., 2018). And, in communities bordering Flint, the probability of legionellosis cases in each census tract correlated to their number of residents who commuted into Flint (Zahran et al., 2018).

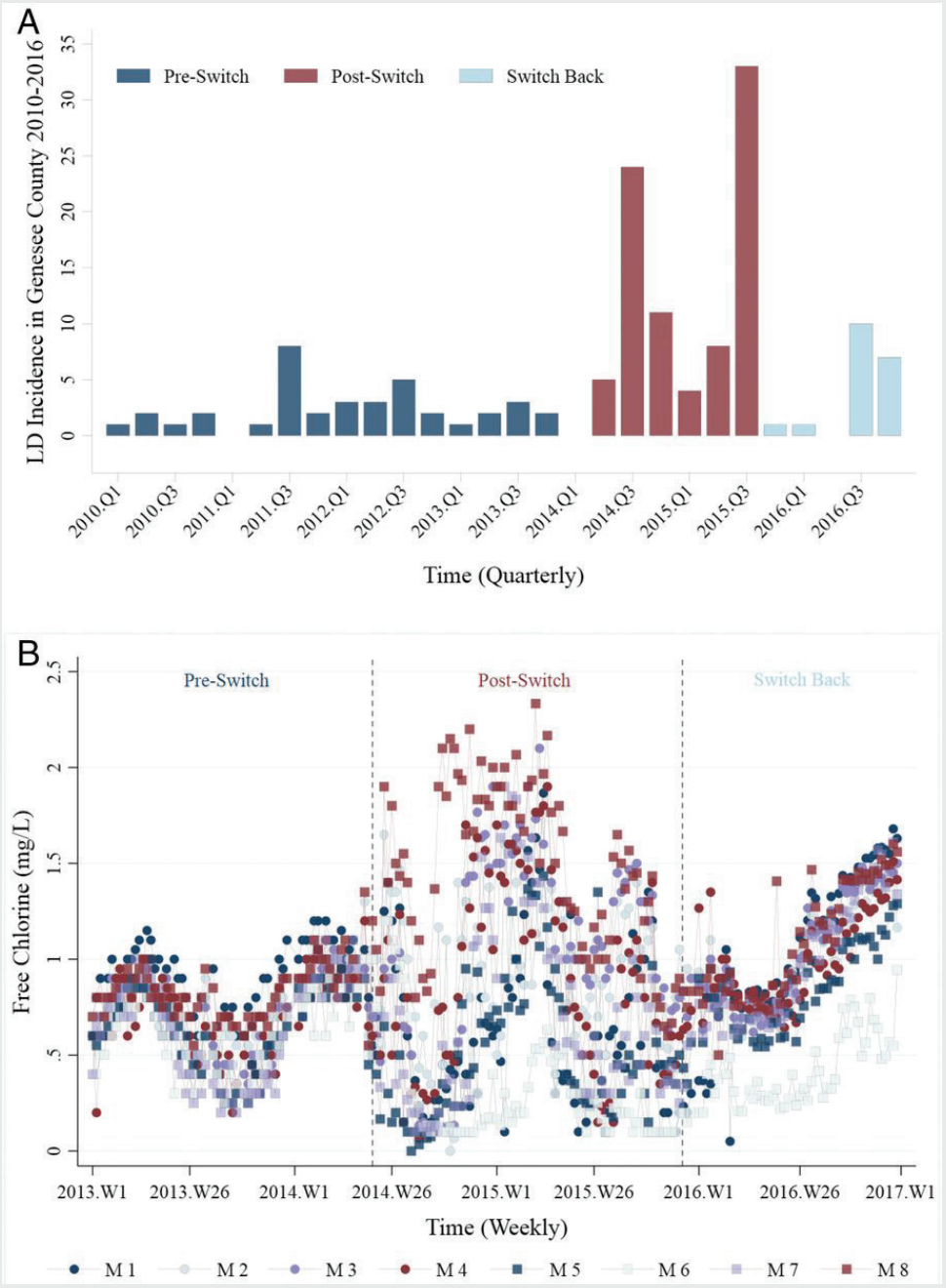

FIGURE 3-9-1 Spike in Legionnaire's disease cases coincident with switch in water supply and increased variation observed in the Flint water distribution system. A. Quarterly Legionnaires' disease incidence in Genesee County, MI, 2010 through 2016. The count of Legionnaire's disease cases in Genesee County as compiled in the Michigan Disease and Surveillance System at the quarterly time step. B. Free chlorine at eight monitoring locations in Flint's water distribution system, 2013-2016. Free chlorine (mg/L as Cl2) was reported weekly during the three water regime phases defined above (vertical lines) and the periods and dates (year/week) shown at eight locations in Flint. SOURCE: Zahran et al. (2018).

Water Quality Monitoring. In addition to inadequate corrosion control of Flint River water, multiple water parameters conducive to *L. pneumophila* persistence or growth were reported. These included slightly elevated distribution water temperature, elevated organic matter, high iron concentrations, and elevated or depleted chlorine residual (Figure 3-9-1B; Masten et al., 2016; Rhoads et al., 2017; Zahran et al., 2018). Iron, an essential nutrient for *L. pneumophila* (Mengaud and Horwitz, 1993), also inactivates chlorine. Indeed, during the period Flint received corrosive river water, as the concentration of free chlorine in water in a census track declined, the probability of Legionnaires' disease cases in that sector increased (Zahran et al., 2018).

Microbiological Monitoring. Water samples collected in October 2015 from the cold-water taps of public restrooms in two Flint hospitals (Schwake et al., 2016) contained *Legionella* and *L. pneumophila* gene copy numbers considerably higher than those reported previously for U.S. drinking water systems in the absence of a legionellosis outbreak (Donohue et al., 2014) (mean concentration range of 1,170 and 2,480 versus 2 gene copies/mL).

In fall of 2016, after Flint had switched back to Lake Huron water, a surveillance study of 130 residences cultured *L. pneumophila* from 13 Flint homes. Of the 16 *L. pneumophila* strains isolated from premise plumbing, one was serogroup 1, and the rest were serogroup 6 (Byrne et al., 2018). In contrast, all 33 clinical isolates submitted from 2013 to 2016 to the Michigan Department of Health and Human Services Bureau of Laboratories by hospitals in southeast Michigan were *L. pneumophila* serogroup 1 (Byrne et al., 2018), consistent with widespread diagnosis by the urinary antigen test.

Conclusions. During the time that Flint River water was used as the city's primary source, corrosion within the Flint municipal water system created conditions favorable for *Legionella* persistence and proliferation. *L. pneumophila* strains naturally present at low levels within the Flint distribution system and building premise plumbing would likely thrive with the influx of organic carbon and iron and the concomitant drop in free chlorine characteristic of the corrosive treated Flint River water. Unfortunately, the small number of clinical and environmental *L. pneumophila* isolates collected during the 2014-2015 outbreak limited the molecular epidemiology attempts to identify the source(s) and parameters that accounted for this outbreak. Only 11 patient isolates were collected in Genesee County between 2014 and 2015 and available for whole genome sequencing, and only eight of these were associated with any exposure in Genesee County during the outbreak. This emphasizes the importance of collecting clinical isolates for tracking potential sources of disease. It should be noted that if the Flint hospitals where patients were likely exposed to *Legionella* had had effective water management plans including on-site controls of *Legionella*, there likely would have been very different outcomes in terms of patients contracting Legionnaires' disease. Indeed, disease cases stemming from one hospital in Flint decreased dramatically after a biocide system was installed in the hospital. Nonetheless, while the Flint outbreak is an example of the failure of an important barrier—treatment of the building water system—it is also unique in highlighting the role of drinking water utilities in creating conditions conducive to *Legionella* proliferation in premise plumbing.

Wastewater treatment plants that service wood-, plant- or food-processing industries in Denmark, Finland, Sweden, and the United States have also been identified as a source of *L. pneumophila* (Castor et al., 2005; Gregersen et al., 1999; Kusnetsov et al., 2010). At these locations, only workers at the treatment plants became ill with Legionnaires' disease or Pontiac fever. *L. pneumophila* at relatively high concentrations (10^7 to 10^9 CFU/L) was mainly observed in sludge and effluent at these plants.

Two recent separate outbreaks of Legionnaires' disease in The Netherlands were traced to biological wastewater treatment plants that treat animal waste (Loenenbach et al., 2018; Alvin Bartels, Dutch National Institute for Public Health and the Environment, personal communication, July 2018). *L. pneumophila* was observed in high numbers (10^6 to 10^8 CFU/L) in their aeration ponds, which contain nutrient-rich water and operate at 35°C. Genotyping of the *L. pneumophila* strains demonstrated that the same sequence type (ST 1646) was observed in patients and in the treatment plant aeration ponds (Loenenbach et al., 2018). Box 3-10 describes the investigation of a Norwegian outbreak of Legionnaires' disease attributed to a wastewater treatment plant.

BOX 3-10
Wastewater Treatment Plant Identified as a Source for Legionnaires' Disease

A wastewater treatment plant in Norway was identified as a source for Legionnaires' disease, leading to 56 cases and ten deaths in 2005, and five cases and two deaths in 2008 (Borgen et al., 2008; Nygård et al., 2008). This plant treated wastewater from a wood refinement factory using both an air-treatment process (air scrubber) and a biological treatment process (aeration ponds). In the air-treatment process, process air was mixed with fresh air before it entered the air scrubbers, where it was sprayed with water. In the biological treatment process, microbial degradation of organic substances from the wastewater was achieved in two large aeration ponds (30,000 m^3 of liquid), and the effluent of the plant was discharged to a river (Olsen et al., 2010).

In 2005, the same genotype of *L. pneumophila* serogroup 1 was observed in patients and in water sampled from the air scrubbers and from a river sample downstream of the wastewater treatment plant (Nygård et al., 2008). Since the water temperature in the air scrubbers was approximately 40°C and it expelled greater than 4 m^3 of water per hour as aerosol and was never disinfected, initially the air scrubbers were the suspected source for the outbreak in 2005 (Nygård et al., 2008). However, despite the control measures taken for the air scrubbers, a second outbreak linked to the same plant occurred in 2008 (Borgen et al., 2008). Additional research showed that *L. pneumophila* serogroup 1 was present in high numbers in the aeration ponds (10^8 to 10^{10} CFU/L) and in the effluent of the plant (up to 10^6 CFU/L) that was discharged in the river (Olsen et al., 2010). In river water downstream of the treatment plant (up to 1.6 km downstream), *L. pneumophila* serogroup 1 was detected at 10^4 to 10^6 CFU/L. The *L. pneumophila* strains were genotyped, and the same sequence type isolated from the patients in 2005 and in 2008 was also observed in the aeration ponds and the river. Moreover, air samples taken above the aeration ponds were consistently positive for *L. pneumophila* by PCR, and cultivation detected up to 3,300 *L. pneumophila* CFU/m^3 air (Blatny et al., 2008). Air samples taken upwind of the aeration ponds were generally negative for *L. pneumophila*, but downwind samples were regularly positive. Therefore, the aeration ponds (and not the air scrubbers) were identified as the primary source of the 2005 and 2008 outbreaks (Olsen et al., 2010).

Occurrence Summary

The vast majority of studies reviewed for this chapter reported presence/absence data but not quantitative concentration data, making it difficult to draw meaningful conclusions about the extent of *Legionella* risk from built water systems. Nonetheless, the preceding section makes it clear that over the 30 years that *Legionella* data have been gathered, the percent positives and concentrations found have not changed significantly over time or with building or device type. Thus, whether large-scale surveys examine cooling towers, residences, hotels, or hospitals, between 30 and 80 percent of the samples are positive for *Legionella* species and 3 to 20 percent are positive for *L. pneumophila*.

The more limited set of studies for which concentrations were reported demonstrates that higher concentrations of *Legionella* are associated with higher disease risk. For example, the studies of *Legionella* outbreaks associated with cooling towers suggest that duration of the outbreak, but not the total number of cases, is related to *Legionella* concentrations averaging greater than 10^6 CFU/L (Walser et al., 2014). One small study in Flint, Michigan, showed positivity levels in hospital taps dropping from 55 percent to 2 percent for *L. pneumophila* along with concentrations dropping from 10^6 CFU/L to below detection limits once the outbreak subsided. Similarly, in two Flint hospitals there was a drop from 80 percent to 40 percent positivity for *Legionella* spp. (with no drop in concentrations) after the outbreak (Rhoads et al., 2017). Non-detectable CFU/L is possible in hospital taps as shown by data obtained from a major hospital's 11-year monitoring program (see Box 3-8).

A number of the studies cited in this chapter included environmental monitoring that recorded concentrations of culturable *Legionella*. The Walser et al. (2014) review of cooling tower outbreaks from France, Germany, Italy, New Zealand, The Netherlands, Norway, Spain, and the UK reported *Legionella* concentrations (for nine outbreaks) ranging from 2.0×10^3 to 1.0×10^8 CFU/L with an average of 1.39×10^7 CFU/L. Leoni et al. (2018) evaluated nine recreational outbreaks of Pontiac fever and Legionnaires' disease associated with hot tubs and bathhouses. Their work reported *Legionella* concentrations ranging from 8.4×10^4 to 1.6×10^6 CFU/L with an average of 8.0×10^5 CFU/L. An outbreak associated with a wastewater treatment plant showed that *Legionella* concentrations from the aerators ranged from 2.0×10^6 to 2.2×10^9 CFU/L with an average of 1.1×10^9 CFU/L (Loenenbach et al., 2018). Finally, Orkis et al. (2018) reviewed data from sporadic cases of disease from several environments (e.g., apartments, homes, high rises, and associated showers and storage tanks) and reported a range of 1.0×10^4 to 2.0×10^5 CFU/L with an average of 1.0×10^5 CFU/L. These data were contrasted to routine sampling concentrations of *Legionella* from reclaimed water, residential properties, hotel showers, and industrial cooling towers (Codony et al., 2002; Johnson et al., 2018; Li et al., 2015; Papadakis et al., 2018). The results are graphed in Figure 3-10. The goal of this exercise was to see if there was an obvious break in the data between sporadic cases and outbreaks, similar to an analysis done for *Cryptosporidium* (Haas and Rose, 1995). The Committee identified the concentration of 5×10^4 CFU/L as such a break. Hence, **a *Legionella* concentration of 5×10^4 CFU/L should be considered an "action level"—that is, a concentration high enough to warrant serious concern and to move remediation forward immediately. A lower action level may be necessary to protect those at higher risk for legionellosis such as hospital patients, particularly those in intensive care, cancer, and solid-organ transplant units.**

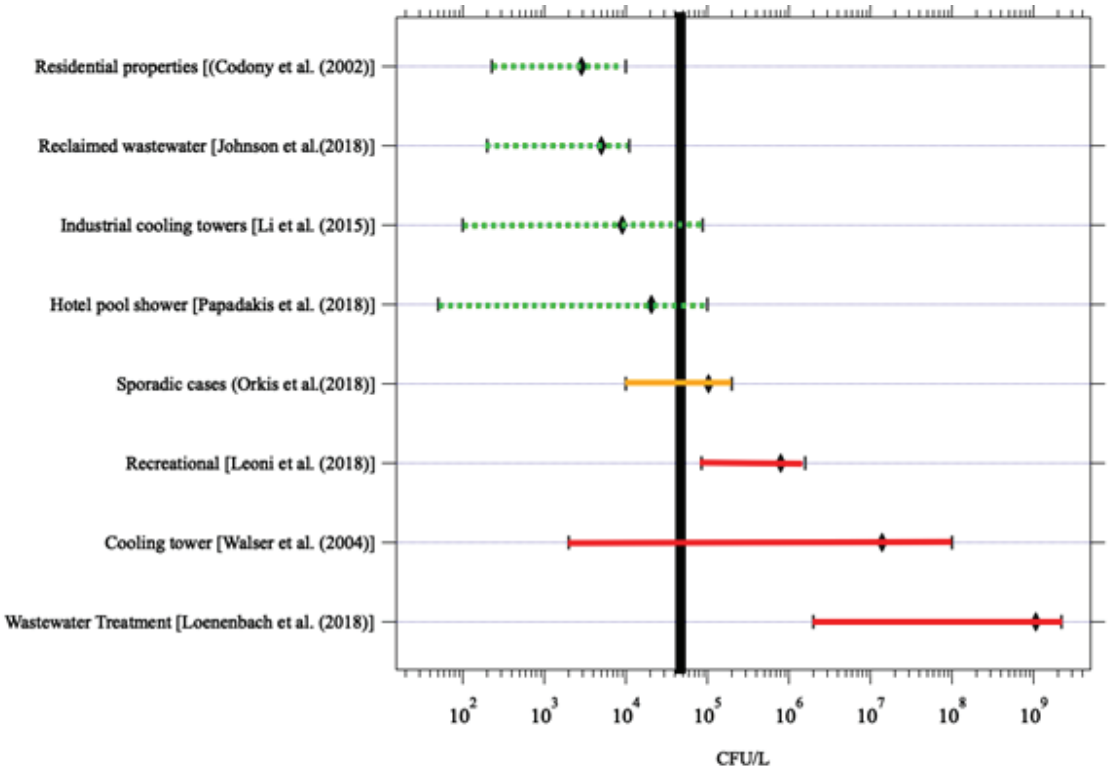

FIGURE 3-10 Concentrations of culturable *Legionella* during outbreaks and routine monitoring from various environments (ranges shown as bars, averages shown as diamonds). Red solid lines are outbreaks. Green dashed lines are routine sampling. The orange solid line is from sporadic cases. The solid black line is the 5 x 104 CFU/L action level identified by the Committee as a break between sporadic cases and outbreaks.
SOURCES: Cooling towers outbreaks (Walser et al., 2014), recreational water outbreaks (Leoni et al., 2018), wastewater treatment plant outbreaks (Loenenbach et al., 2018), sporadic cases from buildings (Orkis et al., 2018), reclaimed wastewater (Johnson et al., 2018), residences (Codony et al. 2002), showers at hotel pools (Papadakis et al., 2018), and industrial cooling towers (Li et al., 2015).

QUANTITATIVE MICROBIAL RISK ASSESSMENT FRAMEWORK FOR *LEGIONELLA*

Quantitative microbial risk assessment (QMRA) is the process whereby the risk associated with exposure to pathogens is assessed (Haas et al., 2014). It evolved from the National Academies of Sciences, Engineering, and Medicine's framework on risk assessment (see Box 3-11), which focused on chemical and physical environmental hazards. QMRA can also be used to assess the Legionnaires' disease risk from exposure to waters containing *L. pneumophila* under various scenarios (e.g., aerosols from toilets, showers, or cooling tower drift).

Risk assessment has multiple applications in understanding and controlling problems from *Legionella*. For example, given an acceptable level of risk in a particular venue or application (e.g., hospital showers, cooling towers), one can use QMRA to estimate the concentration of *L. pneumophila* in the breathing zone (or ultimately, in the water being aerosolized) that would result in that risk. This concentration could be used as a standard, criterion, or operational target to which one would compare the results of routine environmental sampling for *Legionella* to determine whether it is necessary to remediate a building water system and to what extent (i.e., the "how clean is clean" problem). This does not imply

BOX 3-11
Risk Terminology and Definitions

Kaplan and Garrick (1981) set forth the concept of risk as the likelihood of a consequence from a hazard, with attendant uncertainty and variability. As delineated in a framework set forth by the National Academies of Sciences, Engineering, and Medicine (NAS, 1983), human health risk assessment involves the delineation of a hazard, assessment of exposure, determination of the dose-response relationship and aggregation in a risk characterization. These terms are formally defined as:

Risk: The potential for realization of unwanted, negative consequences of an event (Committee on Foundations of Risk Analysis, 2015).

Hazard identification: The process of determining whether exposure to an agent can cause an increase in the incidence of a health condition (NAS, 1983).

Dose-response assessment: The process of characterizing the relation between the dose of an agent administered or received and the incidence of an adverse health effect in exposed populations and estimating the incidence of the effect as a function of human exposure to the agent (NAS, 1983).

Exposure assessment: The process of measuring or estimating the intensity, frequency, and duration of human exposures to an agent currently present in the environment or of estimating hypothetical exposures that might arise from the release of new [agents] into the environment. In its most complete form, it describes the magnitude, duration, schedule, and route of exposure; the size, nature, and classes of the human populations exposed; and the uncertainties in all estimates (NAS, 1983).

Risk characterization: The process of estimating the incidence of a health effect under the various conditions of human exposure described in the exposure assessment. It is performed by combining the exposure and dose-response assessments. The summary effects of the uncertainties in the preceding steps are described in this step (NAS, 1983).

Risk management: Risk management is the process of weighing policy alternatives and selecting the most appropriate regulatory action by integrating the results of risk assessment with engineering data and with social, economic, and political concerns to reach a decision (NAS, 1983).

conducting QMRA for each situation, but rather developing a generic QMRA for types of buildings or exposures to develop actionable cleanup targets (e.g., cleanup such that the average of ten air samples does not exceed a certain value).

Another application of QMRA is outbreak investigations. In this situation the plausibility of a particular source being the cause of an outbreak can be determined by back-calculating the *Legionella* concentrations that would have been there if in fact that site was the cause. There are many other applications of QMRA in the design or remodeling stage of a building. QMRA can inform design decisions and determine, for example: (1) the length of a shower hose that should not be exceeded to avoid unacceptable amplification of pathogens; (2) the setback distances from populations for large industrial cooling towers; or (3) the adequacy of building-level hydraulic design to maintain acceptable microbial quality. In all the above cases, even in the absence of precise data for all inputs, risk can be calculated by estimating the uncertainties for each input and propagating them through the calculations.

From a mechanistic standpoint, a *Legionella* QMRA can be conducted by going through the series of steps shown in Figure 3-11. Given a recovery-corrected concentration of infectious and viable *Legionella* in water, the aerosol generation rate can be computed. Some enrichment of *Legionella* in the aerosol may occur, since bacteria selectively accumulate at air-water interfaces (Schäfer et al., 1998). The size distribution of bacterial-laden aerosols is important with respect to transport, survival, and passage to the lungs.

Once aerosols of the appropriate size are inhaled, the inhaled dose can be used to determine the risk from the exposure via application of a dose-response model. There are dose-response models for L. pneumophila that have been derived from animal experiments and validated against outbreaks (Armstrong and Haas, 2007a, 2008). These are consistent with the beta-Poisson and exponential models (Haas, 2015), such that there is no "threshold" dose below which zero risk occurs. In other words, for any dose, no matter how small, there is a finite non-zero risk of infection thence illness, since even a single organism can, in some fraction of hosts, multiply to a biologically significant level in vivo.

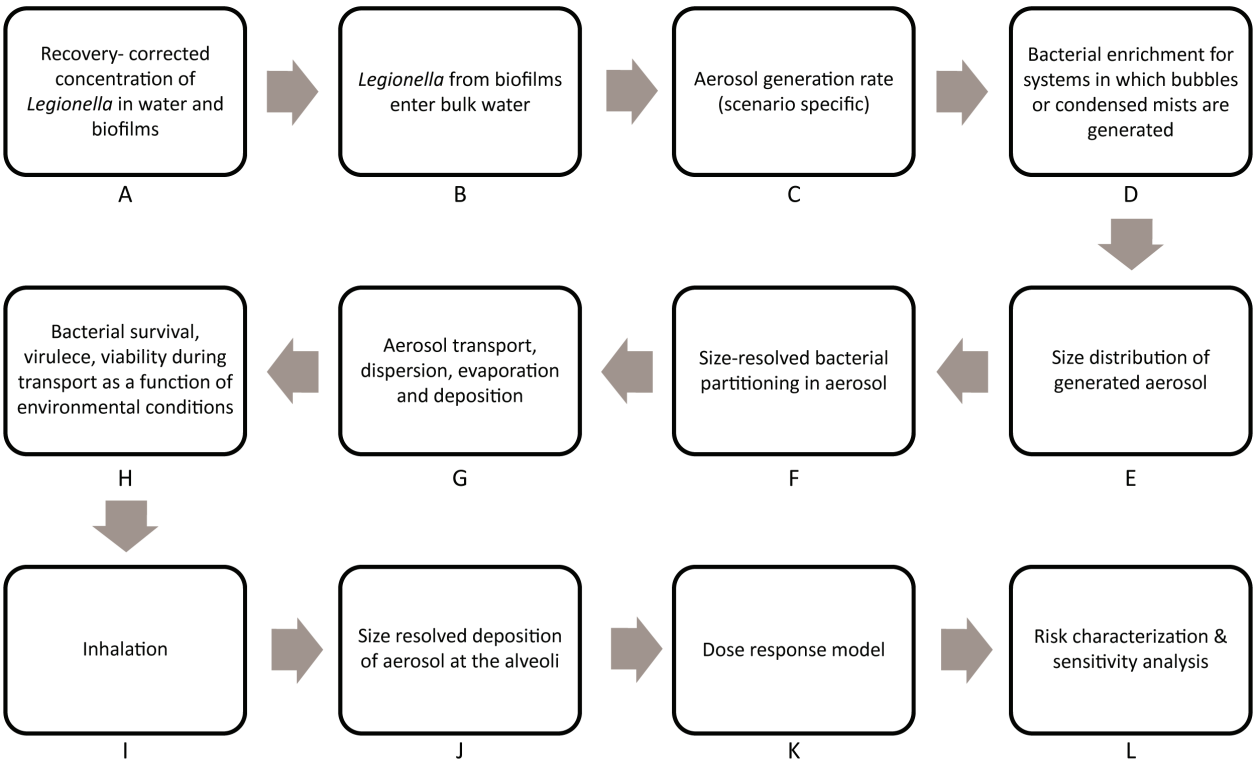

FIGURE 3-11 Framework for *Legionella* QMRA. SOURCE: Revised from Hamilton and Haas (2016). Reproduced with permission of R S C Publications in the format Book via Copyright Clearance Center.

Because the QMRA approach relies on dose-response models from only a few selected strains for which animal testing has been performed, one uncertainty is the incorporation of any strain variability, or variability associated with prior history of bacterial exposure, such as the acquisition or expression of virulence factors (Buse et al., 2015). Also, the current dose-response models are only for L. pneumophila serogroup 1; the relative potency of other serogroups is unknown. The dose-response models do not account for any differences in host characteristics, such as age, gender, or immune status.

If actual data are available for microorganisms at one of the intermediate points in the flow chart, it is possible to start the QMRA at that point. For example, size-resolved microbial concentrations in aerosols might exist which could be used as a starting point (Step F). There have been over 18 exposure assessments and more than ten full risk assessments conducted on *L. pneumophila* (Hamilton and Haas, 2016).

In some cases, concentrations may only be reported as presence/absence. In this situation, concentrations can be estimated using an MPN approach. This is discussed and illustrated in Box 3-12, which indicates that non-detects can be informative *if* the volume examined is known. Non-detects, as well as samples that are "too numerous to count" (TNTC), can also be informative for exposure assessment as long as the volumes examined and the cut-offs for TNTC are known (Haas and Heller, 1988).

Environmental measurements of *Legionella* are frequently made using molecular methods, with qPCR being the most prevalent technique. However, direct sequencing approaches (Timms et al., 2017) may become more common. (A discussion of these and putative viability assays is found earlier in this chapter.) Exposure estimates are necessary to produce good risk estimates, and the number of samples collected in a monitoring program and their detection limits should be sufficient to determine exceedance or compliance with an acceptable risk value. The number of samples can be determined using standard quality control statistics.

As was made obvious earlier in this chapter, the chosen *Legionella* sampling method may influence the measurements of occurrence and concentration. For example, a recent study comparing the concentrations of *Legionella spp.* in wastewater treated for non-potable reuse found dramatic differences between the results from culture, qPCR, and EMA-qPCR methods (Johnson et al., 2018), as shown in Figure 3-12. Culture-based methods generally reported the lowest occurrence and concentrations.

Regardless of the sampling method used for exposure assessment, quantification of any microorganism carries with it many sources of variability. Some variability may be inherent in the time-to-time and place-to-place differences in actual microbial levels, which is irreducible by more sampling. This variability was exemplified by a detailed investigation of hot- and cold-water outlets in nine residential homes and hotels in Cologne, Germany (Völker et al., 2016). The 807 samples taken showed significant variability (up to 4 logs) in *Legionella* spp. concentrations in flushed samples between sampling points within a single building and, for a given point, between hours in a day or between weeks. Other variability may be due to the experimental techniques themselves, including sample collection, concentration, decontamination, processing, and detection. Only a true end-to-end comparison can assess the extent of this intrinsic variability. Such a study requires that a sample be spiked with a known number of organisms and then processed through the entire protocol (i.e., concentration, decontamination, detection) to assess the recovery and its variability. An example of such a study is Bonilla et al. (2015). Sufficient numbers of samples should be taken to make the effect of this intrinsic variability small with respect to the irreducible variability.

BOX 3-12
Using Presence/Absence Data to Estimate Concentrations

Consider that a number (N) of samples, each of volume (V) have been collected. Of these, P samples are found to be positive. The fraction positive is then $f = \frac{P}{N}$. What is the estimate for the concentration (μ) of microorganisms in the system from which the samples were drawn? This is analogous to the single dilution MPN analysis, which has long been discussed (e.g., Cochran, 1950). Under the assumption that the microorganisms in the system from which the samples are drawn are randomly distributed, i.e., Poisson, the following is the best (maximum likelihood) estimate:

$$\mu V = -ln(1 - f) \qquad (1)$$

For many other organisms (although not for *Legionella*), the distribution of microorganisms in water is not random but more heterogeneous than the Poisson distribution (El-Shaarawi et al., 1981; Gale et al., 1997; Pipes et al., 1977). This may be because of intrinsic variability in the environment, or variability in the enumeration, or both. An alternative to the Poisson is the negative binomial distribution with an overdispersion parameter "k"; small k values indicate greater overdispersion, and the limit of k→∞ is the Poisson distribution.

$$\mu V = k\left[(1 - f)^{-1/k} - 1\right] \qquad (2)$$

Figure 3-12-1 is a plot of the estimate for μV given the fraction of samples positive in the case of the Poisson as well as negative binomial distributions with different "k" values. Below a fraction positive of approximately 10 percent, the impact of heterogeneity is negligible. The utility of this approach can be illustrated with a simple example. Suppose 50 mL samples are used and less than 5 percent of them are found to be positive for *Legionella*.

From equation (1), $\mu V = -ln(1 - 0.1) = 0.105$ and therefore $\mu < 0.21/100mL$.

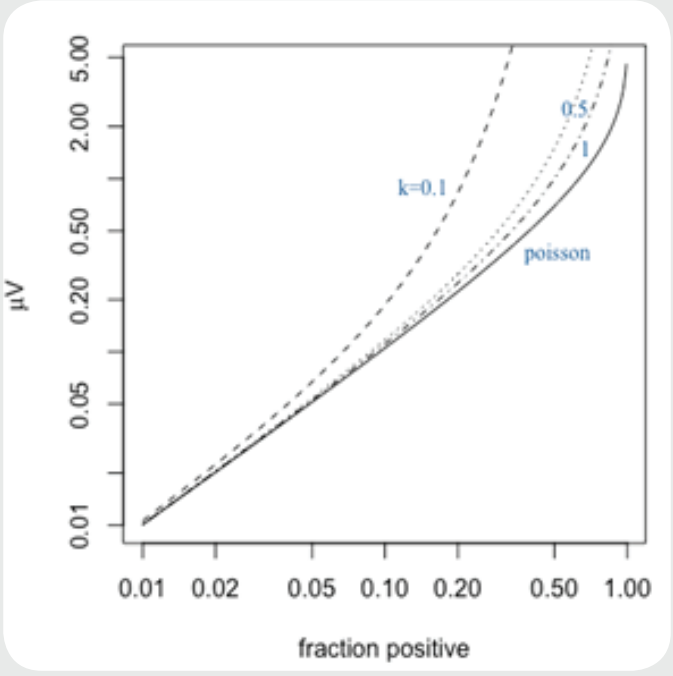

FIGURE 3-12-1 Estimation of Mean Density Given Fraction of Positive Samples of Volume vs. Poisson compared to Negative Binomial Distributions.

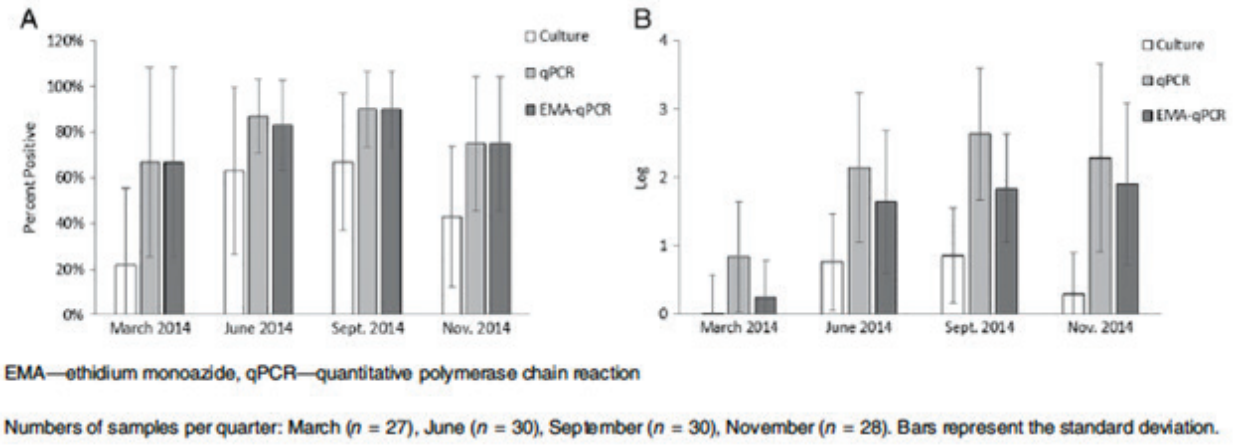

FIGURE 3-12 Seasonal occurrence (A) and concentration (B) of *Legionella* (in CFU/mL for culture, and genomic units per mL for qPCR and EMA-qPCR).
SOURCE: Johnson et al. (2018).

For *L. pneumophila*, there are no good published studies to assess the intrinsic variability of different sampling methods, including culture techniques, molecular techniques, or various proprietary test kits. However, one known factor (albeit with coliforms) is that the variability associated with methods that result in actual concentrations tends to be less than variability associated with MPN-type techniques, although this does depend on how many actual counts are enumerated, and the protocol (number of dilutions and replicates per dilution) of an MPN. As an example, early work by Thomas and Woodward (1955) showed that the MPN enumeration of coliform tended to have about 2.5 times the coefficient of variation for replicates than the membrane filter colony count methods.

QMRA Case Studies for *Legionella*

As an example of a forward QMRA, the risks associated with *Legionella* exposure in aerosols generated from toilet flushing using reclaimed wastewater were examined by Hamilton et al. (2018c). The key inputs required were:[10]

- The concentration of *L. pneumophila*; in this analysis, the monitoring results from several water reuse facilities were used (Johnson et al., 2018) in which *Legionella* spp. were measured using culture techniques, qPCR, and EMA-qPCR, the latter of which is thought to be more closely related to viability (Mansi et al., 2014).
- Measurements of aerosol concentrations in a respirable size range in the vicinity of the toilet after flushing; the size-resolved concentrations from Johnson et al. (2013) were used and aerosols in the range of 1 to 10 μm were considered respirable.
- Respiration rate for light activity of 0.013 to 0.017 m^3/min from the Exposure Factors Handbook (EPA, 2011) was used.

[10] This is designated as "Model 2" in the paper. Three different models, yielding a span of results, were compared.

- Number of flushes per day; a value of 5/d was used (DeOreo et al., 2016).
- Time of exposure to aerosol per occurrence; a range of 1 to 5 minutes exposure per flush was used based on Lim et al. (2015).
- Dose-response relationship for *L. pneumophila* developed by Armstrong and Haas (2007a) from the underlying data of Muller et al. (1983) and Fitzgeorge et al. (1983) were used.

In particular, the first bullet (concentration) has uncertainty because of the issues associated with environmental measurements of viable infectious *L. pneumophila* discussed above. The final bullet (dose-response) has uncertainty because of the use of animal models on a particular strain of *Legionella,* although this has been shown to be consistent with human outbreaks (Armstrong and Haas, 2007b).

Several factors not considered could be of importance. These include the difference between *Legionella* spp. and *L. pneumophila*, the possibility of accumulation of microorganisms at air–water interfaces and thus selective enrichment in the aerosols (Blanchard, 1989), and any inactivation of microorganisms in the period between aerosol formation and inhalation. Based on this analysis, using the three different means of enumerating bacteria in the water (i.e., culture techniques, qPCR, and EMA-qPCR), the annual risks (median) were estimated to be:

- 3.2×10^{-9} (using culture)
- 1.02×10^{-7} (using qPCR)
- 2.56×10^{-8} (using EMA-qPCR)

When compared to a common benchmark of 1/10,000 annual risk, these estimates were substantially lower.

It is also possible to perform a reverse QMRA (Soller et al., 2010), in which the starting point is the desired risk of a scenario (Step L in Figure 3-11); then, the calculations are run "backwards" to ascertain the water quality (Step A in Figure 3-11), aerosol concentration, etc. corresponding to that desired risk. An example of a reverse QMRA is the work of Schoen and Ashbolt (2011), of which a portion is summarized here. They considered the risk of *Legionella* exposure during a single showering event. Starting from a maximum inhaled *L. pneumophila* dose of 1-100 CFU,[11] they considered what the water concentration in the shower might be to attain that level. Key inputs required for their reverse QMRA were:

- Aerosol production rate and microbial partition coefficient (from bulk water to aerosol); values used were based on the experiments of Perkins et al. (2009).
- A respiration rate of 0.012 – 0.025 m^3/min was used (EPA, 2004).
- Size-specific aerosol deposition fractions in the lungs from Schlesinger (1989) were used.
- Duration of exposure in the shower was assumed to be 15 minutes (Perkins et al., 2009).

With this analysis, they computed that a bulk air concentration of 35 to 3,500 CFU/m^3, and a bulk water concentration of 3.5×10^6 – 3.5×10^8 CFU/L would be required to attain the delivered dose.

In all cases, the performance of a QMRA (either in the forward or reverse directions), requires a substantial number of input parameters, each of which may have uncertainty. The resultant risk estimate (or in the case of a reverse QMRA, the exposure estimate) will also not be known with certainty. The calculation of these uncertainties is possible using a variety of techniques, with Monte Carlo methods being the most common.

[11] This would produce a risk unacceptably high in the general population, but was used as an extreme example.

Acceptable Risk

A key question for any QMRA is what level of risk should be regarded as acceptable. This is not (solely) a scientific question, but must be informed by policy, economic, and other social factors. In developing U.S. drinking water regulations for virus and protozoa, the EPA was informed by an annual risk level of 10^{-4} infections/year (Regli et al., 1991). For regulation of carcinogens, a range of 10^{-4} to 10^{-6} cases/lifetime has been used as a range of acceptability (Travis and Hattmer-Frey, 1988). The World Health Organization has widely promoted the use of 10^{-6} DALY/person-year as being acceptable for microorganisms in drinking water (Havelaar and Melse, 2003; WHO, 2008).

The broad community of stakeholders in the *Legionella* arena need to be engaged in a deliberative process to develop acceptability levels in different venues (Renn, 1999). It may be that different venues with different types of exposure and different exposed populations should have different acceptability levels—for example, hospitals with acute susceptible populations and relatively short stays, versus cooling towers with broad, potentially frequent exposure to the general population.

The level of risk that may be regarded as acceptable is associated with the type of hazard (how well it is understood, natural versus human-derived), the consequences (e.g., death) and the ability to control the exposure. For drinking waterborne pathogens, The Netherlands has codified an annual risk of 10^{-4} (1 infection in 10,000 over a one-year time frame). In the United States, for drinking water standards primarily aimed at controlling mild to moderate gastroenteritis, a value of 10^{-4} infections per year is also considered acceptable. However, it is important to examine the daily risk versus an annual risk, as both are incurred every day in the context of drinking water. Annual risk is translated to a daily risk via the relationship below (Haas, 1996):

$$P_{annual} = 1 - (1 - P_{daily})^{365}$$

If daily risk varies day to day, then the annual risk can be computed as follows:

$$P_{annual} = 1 - \prod_{i=1}^{365} (1 - P_i)$$

For an exposure that is relatively continuous to a large population, an annual risk level may be an appropriate approach to control. This could be pertinent to exposures such as large industrial cooling towers. For exposures that may only be short-term, especially to susceptible subpopulations, the control of daily risk could be appropriate. This could be pertinent to situations such as hospitals and nursing homes.

Use of a daily risk level could lead to a different monitoring and control scheme. This is illustrated by the hypothetical Figure 3-13 below. The red line indicates the uniform daily risk that would correspond to 1/10,000 infections per year. The black plot illustrates a random set of daily risks that over the course of the year would result in the same annual risks, despite a high degree of day-to-day variability. For shorter-term exposures, therefore, a population would be exposed to higher risks from time to time rather than to a uniform risk. In the case of *Legionella*, there is a lack of data to know how variable day-to-day exposures, and hence the resultant risks, might be.

In addition to the choice of annual or daily (or some other time period) averaging for assessing acceptability of risk in particular venues, the choice of the endpoint metric needs to be addressed by risk managers. As noted above, both 10^{-4} annual risk of infection and 10^{-6} DALY per person per year have been put forth as useful endpoint metrics in the context of drinking water. These were developed with regard to the risks of gastroenteric pathogens such as enteric bacteria, viruses, and protozoans (e.g., *Giardia* and *Cryptosporidium*). Such organisms have mild to moderate health consequences, such that the 10^{-4} annual risk of infection and 10^{-6} DALY endpoints produce similar results with respect to acceptable microbial quality of water (i.e., the concentration of *Cryptosporidium* in water).

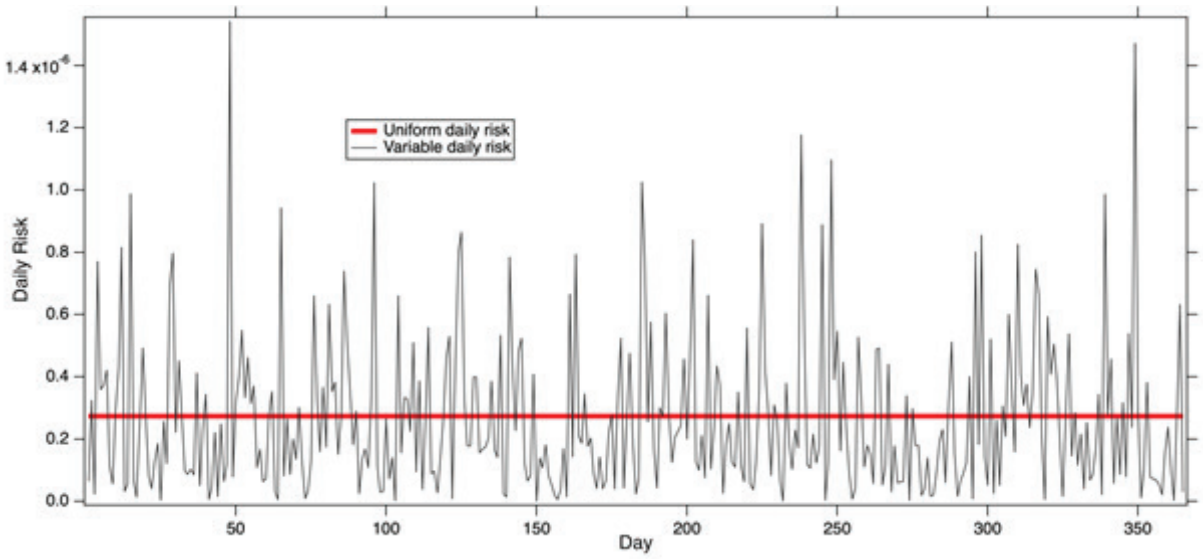

FIGURE 3-13 Sketch of uniform daily risk vs. variable daily risk.

However, the severity of legionellosis leads to a much higher ratio of DALYs per infection as noted in Table 3-9. Compared to cryptosporidiosis, legionellosis is more than 300-fold more consequential. Hence, endpoints of 10^{-4} annual risk of infection and 10^{-6} DALY are not equivalent in this case.

TABLE 3-9 Ratio of DALYs to Infections for Various Pathogens Conveyed Via Water

Illness	DALYs/100 infections
Cryptosporidiosis	0.3
Norovirus	0.3
Salmonellosis	0.3
Hepatitis	17
Legionellosis	97

SOURCE: Abstracted from van Lier et al. (2016).

This is also illustrated in Figure 3-14, in which the water concentrations corresponding to acceptable risk based on per exposure or annually (using either infections or DALYs as the endpoint) are graphed for different types of exposures. In this case, faucet, shower, and toilet exposures using both conventional and water-efficient fixtures are tabulated. Once a risk manager has decided what endpoint metric and acceptability level and what averaging period (if any) are appropriate, then the corresponding water concentration can be determined. For example, if an acceptable annual risk of 10^{-4} has been chosen, then the concentration of *L. pneumophila* measured at a conventional faucet, toilet, or showerhead should be no more than 10^5, 8.6×10^5, and 1.4×10^3 CFU/L, respectively (see Table 3-10). On the other hand, if acceptable risk is based on the 10^{-6} DALY, then the concentration of *L. pneumophila* measured at a conventional faucet, toilet, or showerhead should be no more than 10^3, 8.8×10^3, and 14 CFU/L, respectively. (These numbers are revisited in Chapter 5 as thresholds to help interpret monitoring data.)

Risk management decisions need to be developed for target levels of acceptability to *Legionella* in various settings. While U.S. practice has been to use a 1/10,000 annual infection endpoint as a measure of

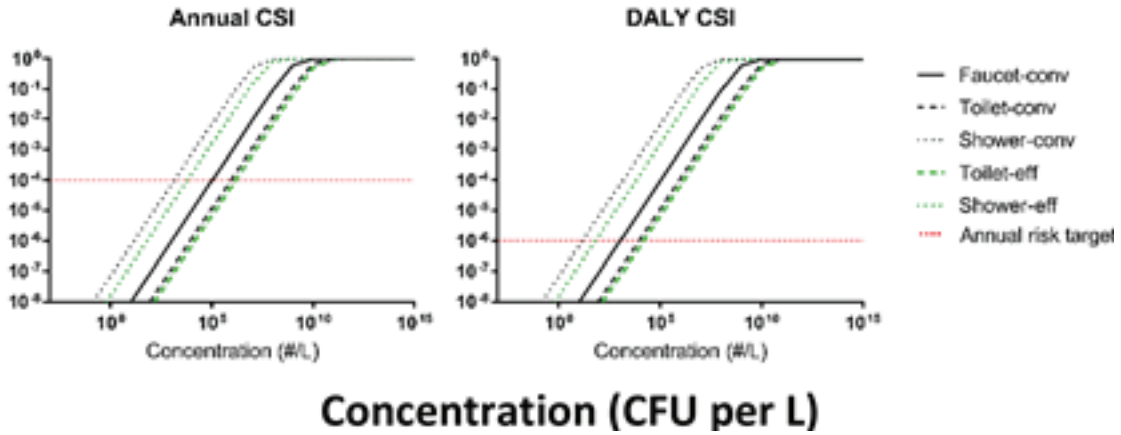

FIGURE 3-14 Curves of *L. pneumophila* concentration versus risk from conventional (conv) and water efficient (eff) fixtures. CSI = clinically symptomatic infection (i.e., disease). SOURCE: Hamilton et al. (2019). https://pubs.acs.org/doi/10.1021/acs.est.8b03000>, and include a notice to readers that further permissions related to the material excerpted should be directed to the ACS.

acceptability in drinking water (Rose et al., 1991), WHO has promoted use of a 10^{-6} DALY annual risk as an endpoint because of the increased severity of Legionnaires' disease. Which risk target is more appropriate, and whether an annual or a daily (or some other time period) average is more appropriate, are specific questions that need to be addressed by risk managers.

The examples above focused on exposure to aerosols in the indoor environment from plumbing fixtures. It is also possible to conduct a QMRA for exposure to cooling towers and other aerosols in the outdoor environment (see Hamilton et al., 2018c), but these circumstances require much more site-specific information. This includes (1) characteristics of the cooling tower, including aerosol generation rate and height, (2) the concentration of *L. pneumophila* within the water producing the aerosol, and (3) wind direction (relative to exposed population), velocity, and meteorological conditions (atmospheric stability).

TABLE 3-10 *L. pneumophila* Concentrations in Various Plumbing Fixtures that Correspond to Target Risk Levels.

Devices/Fixtures	Critical Average Concentration (CFU/L)
Target Risk Value: 10^{-4} infections per person per year	
Conventional faucet	104,000
Conventional toilet	857,000
Conventional shower	1,410
Target Risk Value: 10^{-6} DALY per person per year	
Conventional faucet	1,060
Conventional toilet	8,830
Conventional shower	14.4

NOTE: Median estimates from a Monte Carlo simulation.

SOURCE: Hamilton et al. (2019).

How to Respond to Data and Information Generated from Sampling

The role of liability in the control and prevention of Legionnaires' disease has been mixed in the United States. Multi-million-dollar lawsuits are not uncommon for Legionnaires' disease when the environmental source is tracked to a large building or other entity where the owner and/or other persons are responsible for the safety of those served by an implicated water system. Manslaughter charges have been filed on rare occasions. To protect their clients, some lawyers have advocated that the water facilities considered at-risk (e.g., hotels, hospitals) test their water for *Legionella* as part of a water management plan, while others have advocated that it is better not to test since results could potentially be used against their client. This latter argument will probably not become entrenched as testing becomes more common, and "not knowing" may hurt rather than help the defense. The growing number of litigants and large size of settlements may result in the insurance industry pushing many clients with water systems serving the public into improving their prevention program for legionellosis.

A challenge inherent in implementation of *Legionella* control programs by healthcare centers, assisted-living facilities, hotels, and other commercial buildings, as well as public water supplies, is balancing professional or commercial responsibility with notification of the public when disease cases or water system contaminations occur. No guidelines currently exist, for hospitals or other building management or municipalities, on how to release information when *Legionella* and Legionnaires' disease are detected. Such guidelines are critically important, given the need to provide accurate, actionable information to the public, while protecting patient confidentiality, and taking resource limitations for the entities involved in releasing information into account. CSTE's Legionnaires Disease Surveillance Working Group plans to focus on risk communication, including notification and disclosure, with regards to Legionnaires' disease outbreak investigations and will be coordinating with other relevant national organizations, including the CDC, in the coming months (Monica Schroeder, CSTE, personal communication, April 26, 2019). A policy framework for risk communication should be developed by a coalition of stakeholders, with representatives from infectious disease, epidemiology, microbiology, and public health; healthcare and assisted-living management; hotel and resort management; cooling tower and municipal water management; insurance; liability and privacy law; and ethics. The work of this coalition could be informed by the European Legionnaires' Disease Surveillance Network (ELDSNet); the Public Services and Procurement Canada's *Legionella* Management Communications and Actions Protocol; the federal Sunshine Act to increase transparency in government; and the CDC Foodborne Diseases Active Surveillance Network (FoodNet), a national food safety policy and prevention effort that monitors trends, attributes illness to source, and disseminates information about current foodborne illnesses to the public.

CONCLUSIONS AND RECOMMENDATIONS

This chapter has demonstrated that Legionnaire's disease rates have been rising in the United States and Europe for the past 20 years, and current reported incidence is likely a substantial underestimate of the actual disease burden. **The Committee estimates 52,000 to 70,000 cases of Legionnaires' disease in the United States each year.** There are many sources of *Legionella* risk in engineered water systems, from cooling towers to premise plumbing to hot tubs. Most of the occurrence data gathered from these sources has not been reported as concentrations, making it difficult to discern trends over time and conduct microbial risk assessment. In only a few outbreak investigations have clinical and environmental data been linked to definitively show that a particular water system was the etiological source of disease cluster. The following conclusions and recommendations are made to improve surveillance and diagno-

sis of legionellosis, monitoring of water systems for *Legionella*, and identification of sources of exposure for both sporadic and outbreak-associated Legionnaires' disease.

There is an urgent need to develop better clinical tools that will capture more Legionnaires' disease cases and identify pathogenic *Legionella* beyond *L. pneumophila* serogroup 1. The increasing rates of legionellosis, combined with its associated morbidity and mortality, demand improved diagnostics. First, hospitals in both rural and urban areas should have access to on-site urinary antigen testing to facilitate more targeted antimicrobial therapy and to increase disease recognition. Second, efforts to develop standardized molecular methods for *Legionella* diagnoses (including non-*pneumophila* species and *pneumophila* serogroups other than serogroup 1) should be prioritized by research laboratories and federal agencies. Such methods could increase understanding of the extent of the underestimate of reported disease rates and should be accessible outside of research and academic institutions. Finally, the U.S. Department of Health and Human Services should fund multi-center prospective studies of clinical respiratory samples using these new assays to better understand prevalence and diversity of the *Legionella* species and serogroups causing clinical disease. Once the "true" diversity of human-infectious legionellae is identified, a range of environmental niches could then be explored to identify isolates representing genotypes by niche and preferred methods for their identification from environmental samples. There is also a need for education and a cultural shift from empiric treatment to use of available and future diagnostic tools for *Legionella* to better characterize the true incidence of legionellosis in the community.

The CDC should strengthen the (soon-to-be-merged) NNDSS and SLDSS to include environmental exposures as feasible, including both the potential exposure setting and the type of related building water systems. Although all cases will not receive thorough environmental investigations, at a minimum it should be discerned whether a case may be associated with a healthcare facility, accommodation site, hot tub or other well-recognized potential source, as well as some information about the building water system and any known deficiencies (e.g., water main breaks) during the incubation period. Similarly, within NORS, the CDC should consider housing *Legionella* outbreak data in a separate database from enteric pathogens to make NORS more useful for legionellosis prevention and control. In addition, timely analyses by setting and type of water system, with more frequent updating of publicly available data, would improve the usefulness of NORS for assessment of *Legionella* prevention efforts.

An improved understanding of sporadic, community-acquired cases of Legionnaires' disease is critical to reducing the rising rates observed over the last 20 years. **Determining the most common sources of sporadic disease will require well-funded, population-based studies in multiple jurisdictions (e.g., cities, counties, states).** Such studies would require the recruitment of multiple medical centers with an adequate number of *Legionella* cases each year, willingness and capacity to collect clinical samples for *Legionella* culture, environmental personnel with knowledge of how to sample the most likely sources of exposure for legionellosis patients, and laboratory capacity to reliably grow *Legionella* from clinical and environmental samples. In the United States, clinical cultures are currently available for less than 10 percent of cases; thus, an effective study would have to dramatically improve on the current capacity to obtain cultures from patients. Enhanced clinical culture capacity is also essential to accurately assess the contribution to disease from non-*pneumophila Legionella,* and *L. pneumophila* that is not serogroup 1 (recommended above).

The CDC should work with states to gain closer to real-time reporting and investigation of travel-associated cases. Many outbreaks of travel-associated disease can be best detected at the national

level, since many of the patients who report staying in a hotel or other accommodation during the incubation period have crossed state lines. Currently, reporting of travel-associated cases from many states is neither timely nor complete. Better understanding travel-associated cases is an easy target for intervention, as these data are often readily available from patient interviews, can help to link individual cases to larger clusters, and may help to identify opportunities to limit further exposures.

Although additional *Legionella* program efforts are underway in some states, these efforts are not comprehensive, and most state health departments are severely lacking (both in resources and expertise) in their programs of surveillance, prevention, and control for Legionnaires' disease. **Regional Centers of Excellence for prevention and control of legionellosis could serve as a backbone to strengthen the capacity of state health departments to detect and investigate cases of Legionnaires' disease.** These centers could be modeled on the Integrated Food Safety Centers of Excellence and the Centers of Excellence for Vector Borne Diseases, with modifications to include the relevant disciplines needed for *Legionella* applied research and control. The Centers could undertake critical applied research (e.g., optimizing culture methods and comparing them to new methods and coordinating the in-depth, multiple-jurisdiction studies of environmental exposures recommended above). By building a cadre of experts in *Legionella* prevention and control that includes industrial hygienists and engineers, these centers could promulgate best practices for prevention and control measures (see Chapter 4). Finally, these Centers could train and assist building managers as they create water management plans, and they could initiate certification programs for those responsible for the safety of water systems in built environments (see Chapter 5).

A systematic study to compare culture methods for *L. pneumophila* (and other pathogenic legionellae) with qPCR, viability-qPCR and RT-qPCR is needed to determine comparability. qPCR and its variants offer a more rapid method to quantify *Legionella* in the environment and could be used consistently to inform decisions on decontamination and restoration of affected systems, to investigate the bacteria's ecology and exposure pathways, and as a quality control method. Yet, there are few comparisons of methods, and a better sense of real world performance under "normal" and "bloom" conditions is needed. There are reasons why culture techniques may underestimate the true *Legionella* risk (e.g., VBNC cells) whereas qPCR might overestimate risk (due to response to nucleic acid in nonviable organisms). Whether use of viability qPCR or RT-qPCR could balance these issues in unknown. With side-by-side comparisons of methods in a broad range of settings, it may be that PCR-based or other simplified methods or test kits could be shown to be useful predictors of human health risk and adequacy of remediation.

By reviewing dozens of *Legionella* studies on various building types from around the world, the Committee found the ***Legionella* occurrence data to be highly variable and sparse, making comparisons among studies difficult and detection of spatial and temporal trends almost impossible.** The available data suggest that cooling towers, hot tubs, showers, and wastewater treatment plants can be hot spots for growth of *Legionella* and exposures. This data set could be improved by adopting standardized molecular methods that allow for greater quantitation and more rapid results. Improved environmental monitoring methods could facilitate a temporal and spatial assessment of changes in *Legionella* levels within buildings in several special studies to better understand background levels, potential exposure, and ultimately risk. Finally, a collaborative, widespread national survey of *Legionella* that included distribution systems, premise plumbing in various types of buildings, and cooling towers would be useful for further understanding the concentrations of concern and the risks of sporadic Legionnaires' disease.

The Committee's analysis of studies on *Legionella* occurrence that collected concentration data suggests that a ***Legionella* concentration of 5 x 10^4 CFU/L should be considered an "action level," that is, a concentration high enough to warrant serious concern and trigger remediation.** This concentration could be used for many purposes, including to set an acceptable risk level for Legionnaires' disease and for regulations and guidelines on *Legionella* management in building water systems (see Chapter 5).

There is a good framework to perform QMRA for various *L. pneumophila* exposures. To strengthen these tools, additional knowledge is needed about the impact of virulence and strain differences, phenotypic alterations in potency and aerosol survival, and generation rate of aerosols from various devices. Data on exposures, especially for cooling towers, are lacking. Also, validation of models for predictive growth of *L. pneumophila* in water systems is required. QMRA has many applications from setting action levels for the occurrence of *L. pneumophila* in different venues or targets for remediation to informing design and permitting decisions about pipe length, setback distances for large industrial cooling towers, and building-level hydraulic design to maintain acceptable microbial quality. QMRA can be used to determine *Legionella* concentrations in building water systems that correspond to certain Legionnaires' disease risk levels.

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4

Strategies for *Legionella* Control and Their Application in Building Water Systems

This chapter focuses on strategies for *Legionella* control in building water systems. Such controls should ideally begin as early as the design and commissioning phases and subsequently be applied routinely as preventative measures and, when necessary, for remedial purposes, i.e., in response to outbreak or flags raised by monitoring data. A summary of the key strategies for controlling *Legionella* by affecting their growth and survival (or that of their free-living amoebae hosts) is presented first. The real-world application of these strategies for *Legionella* control in building water systems and devices is then described. Table 4-1 summarizes which specific controls are applicable to which building water systems and devices. The chapter also discusses emerging issues, such as potential conflicts among strategies for green building design, water and energy conservation, and more prospective *Legionella* control strategies.

As detailed in the following sections, factors known to influence *Legionella* growth in water systems include temperature, disinfectant type and levels, hydraulic conditions (particularly avoiding stagnation), presence of nutrients, pipe materials, presence of distal devices, and extent of aerosol formation. Many of these factors come into play during the initial building design and commissioning stages, while others can more readily be adjusted in existing buildings. For example, in a building, the pipe sizing, the materials and devices used, and the flow conditions are determined prior to the building's construction and harder to adjust once a building is operating. Factors such as temperature, disinfectants, and distal devices can be more easily adjusted after building construction and during operation. Control of *Legionella* can be based not only on limiting its growth, but also on limiting the opportunities for humans to be exposed, for example by avoiding the formation of aerosols, particularly those of ideal size (less than 10 mm) for inhalation and deep deposition into the lungs. Aerosols can also be diverted, as in the case of drift eliminators on cooling towers, to reduce potential for human exposure. Additional barriers, such as point-of-use size-exclusion filters, can also be considered for immunocompromised or other sensitive populations.

In addition to drinking, potable water is used for other critical services in buildings, especially hot tubs, spas, and Jacuzzis (collectively referred to as hot tubs), cooling towers, humidifiers, decorative features such as fountains, medical equipment, dental units, and ice machines. Although any of these water systems has the potential to grow and transmit *Legionella*, this discussion is limited to the premise plumbing of buildings, cooling towers, humidifiers, hot tubs, and corresponding water supplies, though some of the basic principles apply to other systems as well.

The precise target for *Legionella* control can be quite complex in terms of species, serotypes, strains, and corresponding virulence factors. Notably, some treatments may shift the composition of types and

TABLE 4-1 Overview of *Legionella* Control Strategies and Relevance of Their Application to Building and Water System Types.

Strategy	Building Water Systems			Large Engineered Systems			Other Devices		
	Large Institutional Buildings (page 205)	Green Buildings (page 227)	Households (page 209)	Potable Water Supply (page 200)	Wastewater Treatment (page 204)	Reclaimed Water Systems (page 203)	Cooling Towers (page 211)	Humidifiers (page 214)	Hot Tubs (page 215)
Temperature Control (page 177)	√	X (incentive is to reduce temperature)	√	? (limited options)			? (future possibility)	√	
Disinfection (page 185)	√	√	? (only POU UV devices)	√	? (somewhat limited)	√	√		√
Manage Hydraulics (page 192)	√	X (prone to low flow/stagnation)	√	√		√	√	√	
Nutrient Limitation (page 194)				? (Dutch example)		√		√	
Plumbing Materials (page 195)	√	√	√	? (limited for DS mains)		√	√		
Distal Portion of Plumbing (page 196)	√	X (low-flow faucets used)	√						
Aerosol Control (page 199)	√	√	√		√		√		

NOTES: √ = a strategy has been successfully used in a particular system; ? = a strategy could be partially used in a particular system, but there are noted limitations/considerations; X = a strategy is actually being worked against in a particular type of system; blank boxes are where there is no indication that a strategy can be used in a particular system.

virulence of *Legionella*, which is difficult to assess and not typically measured. This chapter provides information based on the targets that are described in the available literature. Still, it is important to note that the type of *Legionella* detection method will also influence the perception of efficacy of various controls. The majority of well-documented case studies base their evaluation on measurements of *Legionella* or *Legionella pneumophila* using culture-based methods, which cannot detect viable but non-culturable (VBNC-like) forms. Certain control strategies like heat treatment, chlorine-based disinfectants, and copper-silver ionization are known to trigger *L. pneumophila* to enter a VBNC-like state (see Chapter 2, Allegra et al., 2008, 2011).

It is clear from research and practice that, in most situations, "zero" is not an achievable target for evaluating whether *Legionella* has effectively been controlled, for several reasons. First, some level of *Legionella* is common in drinking water systems in the absence of an outbreak. For example, *L. pneumophila* serogroup 1 was detected in nearly half of public and private cold-water taps tested in a national survey, with the mean and median concentrations being 1.97×10^3 gene copes per liter (GC/L) and 62 GC/L, respectively (Chapter 3; Donohue et al., 2014). Second, current human-health risk models indicate that a bulk water concentration much higher than "zero" (see Chapter 3; Perinez et al., 2018; Pourchez et al., 2017) is actionable and associated with transmission of *Legionella* into the lungs. Third, monitoring methods are limited in their ability to assess live cells and are subject to detection limits; none can confirm "zero."

In evaluating any building water system, it is important to recognize that *Legionella* does not exist in isolation, but is part of a complex microbial ecosystem spanning biofilms, bulk water, and aerosols. Thousands of other species of bacteria and other microbes reside in these environments (Chapter 2; Pinto et al., 2014) and can potentially enhance or inhibit the growth of *Legionella* (Paranjape et al., 2019; Wang et al., 2013a). Most notoriously, free-living amoebae play a key role in amplifying *Legionella* and enhancing its virulence; thus, it has been suggested that effective control strategies should also target amoebae (Thomas and Ashbolt, 2011). However, such approaches that potentially tap into more precise control of the microbial ecology of premise plumbing to manage *Legionella* are still in their infancy. Here we seek to provide information about how various controls influence *Legionella* and, where possible, their free-living amoebal hosts.

FUNDAMENTAL FACTORS FOR *LEGIONELLA* CONTROL

Temperature

A fundamental control strategy for *Legionella* in buildings is to keep the hot- and cold-water systems at temperatures outside the organism's growth range of 25°C to 43°C (see Chapter 2). Warm water leaves a water system especially vulnerable to *Legionella* colonization and growth. Several studies summarized in this section, across multiple scales, countries, and building settings, demonstrate the overarching benefit of elevated temperature for *Legionella* control. In particular, water heater settings of greater than 60°C are a key threshold for reducing positive detection of *Legionella* as well as for reducing Legionnaires' disease cases and outbreaks. Adjusting the temperature at the water heater outlet to ensure temperatures greater than 55°C to distal points[1] can be highly effective in reducing the proportion of *Legionella*-positive swabs or water samples (Arvand et al., 2011; Blanc et al., 2005).

Temperature control strategies fall into two broad categories: *preventive* and *curative*. Preventive refers to maintenance of (1) elevated temperatures (greater than 55°C) to limit colonization and growth of *Legionella* across hot-water systems and (2) sufficiently cool temperatures (less than 25°C) across cold-water systems. Curative approaches, on the other hand, are somewhat varied in their application,

[1] "Distal point" refers to the point of connection to a fixture such as a faucet, showerhead, thermal mixing valve, etc. Hence, the distal point is just upstream of the point of use. Temperature measurements at the tap are representative of conditions at distal points unless there is a thermostatic mixing valve.

generally involving elevating the temperature temporarily as a "heat-shock" approach. Heat shocks may be applied one time or many times, for various durations, and over a range of temperatures (60°C to 70°C). It should be noted that eradication of *Legionella* species (spp.) and *L. pneumophila* reservoirs can only be achieved at very high temperatures. Work by Epalle et al. (2015a) shows that only strict thermal treatment (i.e., 70°C for 60 minutes) kills more cells and renders non-infectious all *L. pneumophila* strains, both environmental and clinical, but milder heat treatment shocks (60°C to 70°C for 30 minutes) do not. Recent investigations by Cervero-Arago et al. (2019) suggest that prolonged exposure to high temperature (greater than 60°C) can be efficient against both culturable and VBNC-like cells of *L. pneumophila*, and most importantly, that the loss of culturability after heat exposure is associated with decreased virulence and host infection.

The temperature set at the water heater is not equivalent to the temperature experienced at the tap. One controlled study demonstrated that hot water received in taps can cool to room temperature within 30 minutes (Rhoads et al., 2015a). To counteract this, large institutional buildings, such as hospitals, are required by plumbing codes to have hot-water circulation lines leading from the water heater, throughout the building, and back to the heater. This helps provide hot water on demand in distal reaches of the building and also keeps the water lines sufficiently hot to deter *Legionella* growth. Recirculation lines cannot reach each point of use, such that the volume of water between the recirculating pipe and the faucet or showerhead will remain stagnant between uses. Even with recirculation, temperature losses are expected throughout the piping as a function of water circulation and piping isolation. This can result in large variations of water temperatures at distal points, including temperatures that increase risk for *Legionella* growth (Bédard et al., 2015; Boppe et al., 2016).

None of the control strategies discussed in this chapter occur in isolation, and they all have interactive effects. In the case of temperature, the associated water-use frequency is an important factor in determining the temperature regime experienced at the tap (Rhoads et al., 2015a). Thus, efficacy of temperature control is intimately related to the hydraulics of the system. Figure 4-1 illustrates a standard hot-water system as commonly applied in large institutional buildings, including recirculating options and points where temperature control may be applied. This section focuses on the basic evidence of temperature control efficacy, while later parts of the chapter discuss specific applications in buildings and devices.

Impact of Temperature on Legionella in Building Water Systems

Groothuis et al. (1985) observed that when the temperature of a hot-water return line in buildings is maintained at 60°C, cultivable *L. pneumophila* was not observed, but when the temperature was lowered to 54°C, *L. pneumophila* was culturable. Similar observations have been made by others. *L. pneumophila* could be cultivated from a hot-water system at a hospital that maintained hot water at 43°C to 45°C, but not at a hospital where hot water was maintained at 58°C to 60°C (Plouffe et al., 1983). Apartments in the Chicago area (n = 95) that had water temperatures below 60°C in the premise plumbing were more often colonized with cultivable *L. pneumophila* (42 percent) than were systems with water temperatures above 60°C (7 percent) (Arnow et al., 1985). In a survey of 40 Italian hotels, hot water above 60°C in the drinking water system and above 55°C in the outlet water was protective from legionellae (Borella et al., 2005). Finally, cultivable legionellae were only isolated from drinking water in hotels (n = 385) in Greece when water temperatures were between 23.7°C and 60.3°C (Mouchtouri et al., 2007).

Table 4-2 summarizes several examples of the efficacy of thermal controls in healthcare facilities. The Hungarian study (Barna et al., 2016) in Table 4-2 is particularly illustrative of the overarching importance of thermal control of *Legionella* in hot-water plumbing. Over seven years, 1,809 samples were collected from healthcare facilities (n = 22), accommodation sites (n = 21), educational institutions (n = 26),

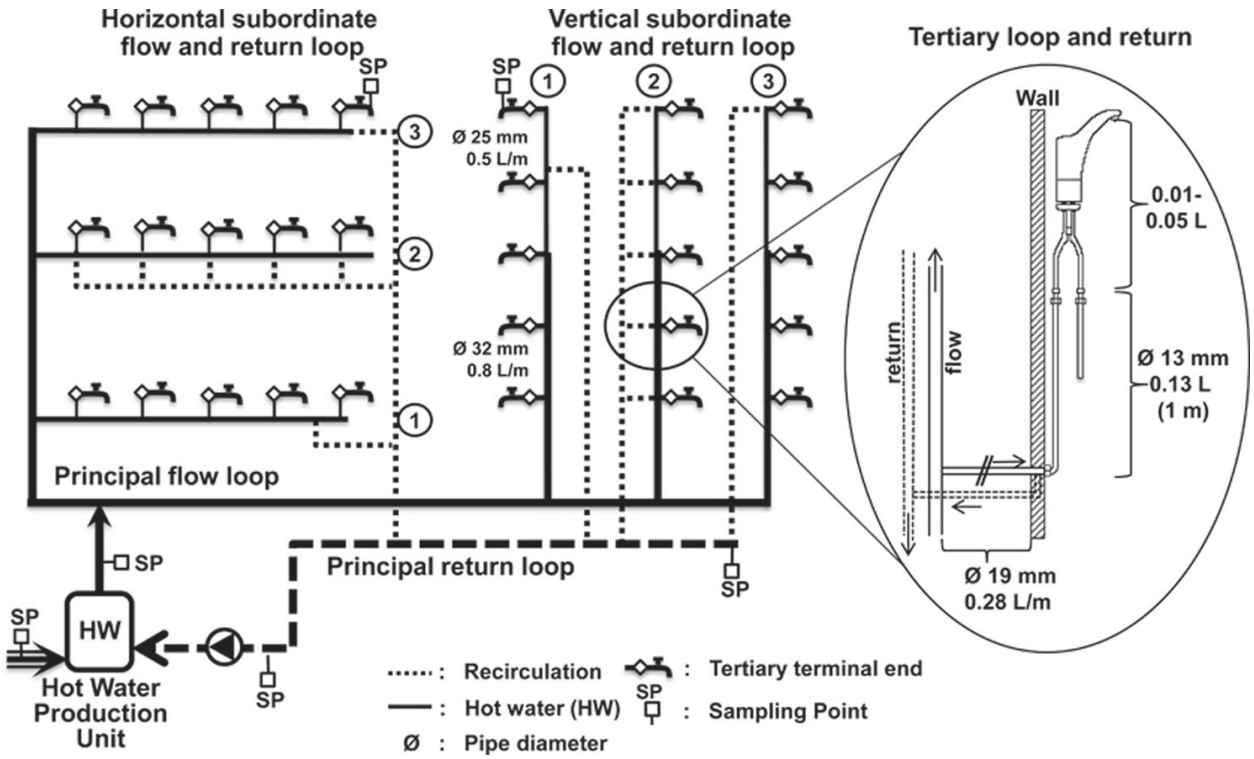

FIGURE 4-1 General schematic of a hot-water system including temperature control points. Three different types of vertical and horizontal hot-water systems are represented: (1) recirculation before the last tap; (2) recirculation connected after each device; and (3) recirculation connected after the last device.
SOURCE: Bédard et al. (2015).

office buildings (n = 10), industrial buildings (n = 35), and private residences with central (n = 26) and individual hot-water supplies (n = 26). Water temperature was found to be the most important factor in a multiple linear regression analysis of 11 system and water characteristics associated with *Legionella*. In general, Table 4-2 and other reports on the efficacy of the implementation of temperature control in healthcare facilities (Bargellini et al., 2011; Lee et al., 2011; Serrano-Suarez et al., 2013) reveal moderate success. Differences among these reports most probably reflect whether the temperature set points were actually reached across the whole system, including at the outlets (e.g., faucets and showers). In most case studies, the actual application of temperature control is poorly documented, with only partial information on temperatures available for the water heater and the return line.

Indeed, thermal control is greatly improved if hydraulic deficiencies are addressed, ensuring that water temperatures greater than 55°C reach distal points, resulting in lower positivity and concentrations of *L. pneumophila* using both culture and quantitative polymerase chain reaction (qPCR) methods (Boppe et al., 2016; Blanc et al., 2005; Lecointe et al., 2018). Bédard et al. (2015) showed that local deficiencies in the hydraulics of hot-water recirculation resulted in lower temperatures and elevated levels of *L. pneumophila*; they correlated these issues to the location where clinical cases of Legionnaires' disease occurred. Heat-shock treatment at 70°C to remove *L. pneumophila* reservoirs and then maintaining temperatures above 55°C at the distal points of a large 1,000-bed hospital were highly efficient at reducing *L. pneumophila* to undetectable levels (using either culture methods or qPCR).

TABLE 4-2 Long-term Healthcare Facility Experience Showing the Importance of Maintaining an Adequate Preventive Thermal Regime to Control *Legionella*

Size of Building(s) Number of Samples Study Length	Key Findings	Reference
Hospital with 870 beds in Switzerland Number of samples unknown 7-year study	• On-site disinfection introduced after regulations lowered the hot-water temperature to 50°C for energy conservation. • Study showed that chemical treatment is not efficient without adequate thermal regime. They operated parallel hot-water systems with (1) copper-silver (Cu-Ag) ionization using 0.6 mg Cu/L and a temperature of 50°C, and (2) ozonation using 0.3 mgO_3/L residual and a temperature of 50°C. • After 3 years, the positivity for *Legionella* spp. remained high in both the ozone-treated networks (65% to 56%) and in Cu-Ag-treated systems (90% to 93%). • After increasing the temperature to 65°C, *Legionella* prevalence decreased to 29% (O_3) and 39% (Cu-Ag). • Increasing temperature was effective in reducing the prevalence and the concentration of *Legionella* spp. in the biofilm swabs. The remaining positive points were associated with temperatures < 50°C because of poor water circulation.	Blanc et al. (2005)
450-bed Swedish hospital with history of *L. pneumophila* nosocomial cases and 1991 outbreak (31 cases)	• Over a ten-year period, *L. pneumophila* prevalence and nosocomial cases of legionellosis were monitored. • Period 1: temperature of hot water at 45°C; outbreak occurrence. Period 2: temperature of hot water set to 65°C with regular flushing (18 months). Period 3: temperature of hot water set to 65°C and > 55°C in the return loop without weekly flushing. • During Periods 2 and 3, positivity decreased from nearly 100% (during Period 1) to 5-23% with remaining low incidence of nosocomial cases (1/year).	Darelid et al. (2002)
18 facilities in the Czech Republic 805 samples	• Highest levels of colonization by *L. pneumophila,* as shown by concentrations in 1-min flush, measured at distal sites where the temperature was 40-45°C. • When temperature was raised to greater than 50°C, positivity decreased. Only non-detects at temperatures over 60°C. • Treatment by shock chlorination (50 mg/L for 2 hours) was less efficient at temperatures of 45-49.9°C.	Hruba (2009)
4 healthcare facilities in Germany 625 samples (316 cold and 309 hot)	• Field studies support the importance of maintaining elevated temperatures at distal locations (estimated by the temperature after 1 minute of flushing), with 4 to 11% of samples positive at temperatures greater than 55°C vs. 14 to 82% positive at temperatures less than 55°C.	Arvand et al. (2011)

TABLE 4-2 Continued

Various building types in Hungary 1,809 samples 7-year study	• Surveyed healthcare facilities (22), accommodation sites (21), educational institutions (26), office buildings (10), industrial buildings (35), and private residences with central (26) or individual hot water supply (26). • Monitoring for *Legionella* over 7 years shows that when hot-water temperature was greater than 55°C, 16% of samples exceeded 10 CFU/L, as compared to 54% for systems with temperatures below 55°C. • For all systems combined, a temperature decrease of more than 10°C within the system led to 62% of samples with concentrations greater than 1,000 CFU/L, compared to 46% for a 5-10°C decrease and 40% for less than 5°C decrease.	Barna et al. (2016)
Pediatric hospital with 450 beds in Quebec 46 samples	• Old hospital with copper piping had very high positivity for *L. pneumophila*. Positivity (%) and concentrations of *L. pneumophila* varied with temperature at the outlets, ranging from no detection at temps greater than 60°C, to 56% positive at temps greater than 55°C, to 100% positive at taps with temps between 40 and 45°C. • Using continuous temperature and flow measurements, areas at risk for lower temperatures were identified. Hydraulic distribution of hot water was improved by balancing return loops and removing dead ends and faulty mixing valves. • Corrective action reduced positivity much more drastically than it did the range of concentration at positive sites.	Boppe et al. (2016)
Tertiary care hospital with 400 beds in Quebec 2 hot water systems 64 samples from hot water system	• Following nosocomial cases of legionellosis, a large hot-water system already using Cu-Ag treatment but with high positivity for *L. pneumophila* (81.5%) was disinfected using heat shock at 70°C. • Temperatures were adjusted from previous set points of 55°C at the water heater and 50°C in the main recirculation pipes (to meet energy conservation goals) to 60°C at the heater with a minimum of 55°C in the return loop. • Extensive temperature and *L. pneumophila* monitoring conducted in the hot-water system and at taps for 2 years. • *L. pneumophila* levels decreased rapidly by culture and more slowly by qPCR, but a significant portion of taps remained positive (20%). The remaining low concentration, positive samples were in areas with hydraulic deficiencies.	Bédard et al. (2016a)
Primary and tertiary hospital with 1,000 beds in France 127 sampling locations 726 samples	• New hospital colonized by *Legionella* spp. (21.3% culture) and *L. pneumophila* (28% qPCR and 1.1% culture) during commissioning, even with a preventive flushing program in place. • Curative and corrective measures over 4 years included (1) thermal shock treatment at 70°C for 30 minutes at each point, and (2) increasing mean distal temperatures from 31°C during start up to 49.1°C. This decreased positivity by 24.1% for *Legionella* spp. by culture with no detectable *L. pneumophila*. • First efforts of hydraulic balancing to ensure greater than 50°C at all distal points decreased positivity further to 2.5%, with remaining positive points where temperature was below 38°C. Improved balancing further increased distal temperatures to 56.3°C, resulting in no positive detection of *L. pneumophila* by both culture and by qPCR for the following 4 years. Low levels of *Legionella* spp. persisted by culture and qPCR, *L. anisa* being the only strain identified.	Lecointe et al. (2018)

The effects of temperature on legionellosis risk are dynamic and intimately connected to the plumbing configuration and hydraulic conditions. Rhoads et al. (2015a) observed that setting the water heaters at a temperature that technically is within the inhibitory range for *Legionella*, in this case 51°C, can actually enrich for *Legionella* in distal pipes. Further, a seemingly simple matter of whether a hot-water pipe is oriented with upward or downward flow can directly affect *Legionell*a levels close to the point of use. Indeed, since cooler water is denser, upward plumbed pipes experience convective mixing, which delivers more nutrients and pushes distal pipes back into the warm-water range conducive to *Legionella* growth (Rhoads et al., 2016b).

Thermal Control in Residential Hot-Water Systems

Residential water systems vary depending on the type of building, with centralized hot water generation being more common in large buildings, often with recirculation. In residences, electric or fuel-heated tanks and on-demand water heaters are commonly used, with a possibility of in-tank recirculation. Balancing the thermal and sanitary performance of domestic hot-water storage is a growing concern as energy stored in sanitary hot-water systems represents about 14.8 percent of total residential energy consumption in the United States[2] and 19 percent of residential energy consumption in Canada.[3]

The type of water heater and the presence of storage and recirculation are critical features in determining the risk of *Legionella* spp. and *L. pneumophila* in residential hot-water systems. Electric water heaters are by design thermally stratified, with lower temperatures found in the bottom section; in contrast, oil and gas water heaters are not stratified because the heating element is located under the bottom of the tank. On-demand water heaters are discontinuous and will deliver water at a set temperature without any storage if properly sized. Many extensive field studies in American, Canadian, Danish, and German residential water systems have demonstrated the prevalence of *Legionella* in hot-water heaters that are thermally stratified (Alary and Joly, 1991; Dewailly and Joly, 1991; Marrie et al., 1994; Mathys et al., 2008; Stout et al., 1992; Wallet et al., 2016). In particular, Dewailly and Joly (1991) investigated 205 electric water heaters using high-volume samples (500 mL) and reported more than 45 percent positivity for *L. pneumophila* serogroups 4 and 2 in the water heater sediments, while no positives were detected in 50 oil or gas water heaters sampled. They identify the major factors for positivity to be the type of water heater (electric versus gas) and the temperature at the bottom of the water heater (less than 40°C). Alary and Joly (1991) observed that 39 percent of the 178 electric water heaters sampled in the Quebec City area were positive for *L. pneumophila* by culture with a wide variety of serogroups present. Despite a relatively high water heater outlet temperature (56.6°C ± 0.4°C) in electric water heaters, 12 percent of faucets and 16 percent of showers were positive. Noteworthy is the fact that no gas- or oil-fired water heaters operated at a higher temperature (61.5°C ± 1.1°C) had distal sites (showers and taps) that were positive for *L. pneumophila*. In a survey of 343 German residential water heaters with a water tank and, in some cases, recirculation, 94 percent of sites were positive for *Legionella* spp. in flushed samples by culture, most (93.7 percent) being *L. pneumophila* (Mathys et al., 2008). No positive sites were detected by culture if a temperature greater than 60°C in the main piping was maintained or if on-demand water heaters producing water with higher temperatures were used. Borella et al. (2004) found that tank size and the distance between the heater and the tap were significant factors in positivity and that different species and serotypes of *Legionella* were associated with different heater types.

Studies have also shown the importance of maintaining high temperatures at the distal ends of hot-water systems. In Germany, an analysis of over 30,000 water samples collected over a period of

2 See https://www.eia.gov/consumption/residential/data/2015.

3 See https://www.nrcan.gc.ca/energy/products/categories/water-heaters/13735.

seven years (2003 to 2009) from 4,600 public buildings for compliance purposes was completed to establish the prevalence of *Legionella* and the conformity of hot-water systems to regulated minimum temperature requirements (Kistemann and Wasser, 2018). Overall, 15.8 percent of all samples were positive for *Legionella*, with positivity highest at distal sites (18.8 percent), lower in the recirculation loop (10.2 percent), and lowest in flushed samples (4.7 percent). More importantly, concentrations were higher by more than an order of magnitude at distal sites, corresponding to lower mean temperatures (47.2°C) versus temperatures found in the recirculation (54.8°C) and in the flushed samples (58.8°C). Figure 4-2 summarizes the impact of water temperature on the percentage of exceedances of the German standard of 100 colony forming units (CFU)/100 mL at distal sites, in the main piping, and in the recirculation loop. In the two lowest temperature classes (up to 45°C), approximately 22 percent of the samples were above the standard in the flushed samples (Vorlauf), 20 percent in the samples from the recirculation loop (Rucklauf), and about 15 percent at distal sites (Peripherie). The situation reverses when temperatures exceed 45°C, with increased prevalence at the distal sites. Even with temperatures at the outlet of 55°C to 60°C after a one minute flush, 5 to 7 percent of the samples remain positive, while fewer positives are found in the flushed and return loop (1 to 3 percent).

Heat Shock

Temporarily elevating the temperature, or heat shock, is applied in a variety of forms and generally is intended as a temporary remedial or emergency measure, not as a preventive measure. An example would be maintaining a water temperature of at least 70°C for at least 30 minutes at each point of use for decontamination of an entire building water system. The efficacy of heat shock is controversial. For example, Temmerman et al. (2006) observed that *Legionella* numbers increased following system recovery from heat shock, presumably because of bacterial growth on nutrients liberated from killed cells (necrotrophic growth).

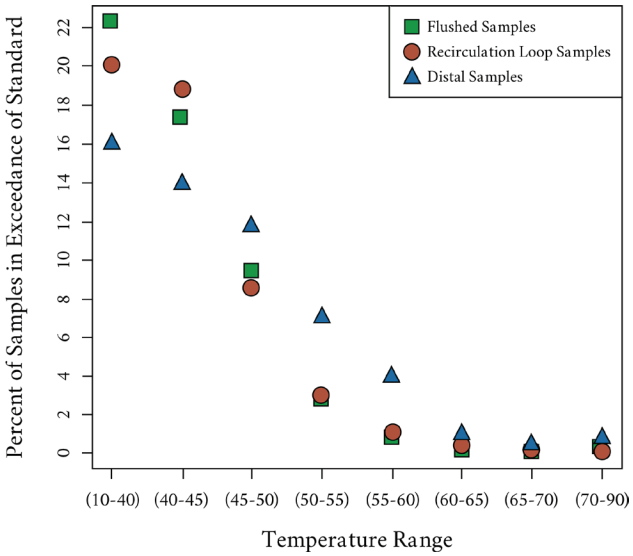

FIGURE 4-2 Relationship between temperature (x-axis) and the percentage of samples exceeding the German standard of 100 CFU/100mL (y-axis) for *Legionella* spp. from public buildings in Germany over a seven-year period. The green squares are for flushed samples, the blue triangles are distal samples, and the red circles are samples from the recirculation loop.

SOURCE: Kistemann and Wasser (2018).

Temperature, duration, and frequency of heat shock application are certainly important factors. The efficacy of a stringent thermal shock (70°C for 30 minutes) on culturable *Legionella* is high in water but limited in biofilms, and most importantly, of short duration (Saby et al., 2005). Moreover, frequent heat shocks can promote the emergence of heat-resistant *L. pneumophila* strains, as observed in hospital water systems submitted to periodic extreme temperature (24 hours at 65°C a few times a year), while no such resistance was observed for strains isolated from the system where heat shock treatments (70°C for 30 minutes) were sparingly applied (Allegra et al., 2011).

Periodic heat shocks at 60°C were compared to a well-managed system continuously maintained at 60°C by analyzing *L. pneumophila* and microbiota in the water plumbing (Ji et al., 2018). Results suggest that maintaining the water system at a set point of 60°C and water use frequency are more promising for the long-term control of both the microbial community and *L. pneumophila*.

Heat shock should be considered as an extreme remediation measure because of such potential problems as (1) the dislodging of particles from piping walls due to thermal shock, which can subsequently cause clogging in balancing valves; (2) damage to equipment from sustained high temperatures; and (3) requirement for close supervision during the process to protect patients, staff, and visitors from scalding. Compatibility of system materials for heat shock is a key consideration. For example, faucets should be designed and constructed with materials that can withstand a superheating treatment. Each component of the system should be evaluated to determine the effect of high water temperatures on materials and equipment (e.g., thermostatic mixing valves). Mitigation measures, such as bypass, should only be considered to protect equipment that cannot withstand the specified temperature and time, since they can themselves become a reservoir for *Legionella*.

Scalding

The higher water temperatures (greater than 140°F/60°C) that prevent *Legionella* growth are associated with an increased risk of scalding and burns. Those at increased risk include young children, elderly patients (older than 65 years of age), and those with substance-abuse disorders, physical disabilities, neurologic illness/disabilities or altered mental status. The U.S. Centers for Disease Control and Prevention (CDC) found that between 2001 and 2006, adults older than 65 years made an estimated 51,700 initial visits to emergency rooms for nonfatal scald burns (CDC, 2009). Over this time period, the average was 8,620 visits per year with an estimated average annual rate of 23.8 visits per 100,000 population. Although most scalding and burn injuries in the homes are related to exposures other than hot water, such as food, cookware, and microwaved items, the risk of scalding from home premise plumbing remains important. It is difficult to tell from CDC (2009) which cases were, in fact, plumbing related. Bathtubs and showers are associated with prolonged exposure to larger body-surface areas, and therefore are particularly concerning for scalding of at-risk populations.

As shown in Table 4-3, scalding and burns are linked to water temperature and time of exposure (Armstrong, 1978; Moritz and Henriques, 1947), as is the growth potential of *Legionella* (Klein, 2018). The CDC, the American Academy of Pediatrics, the American Society of Sanitary Engineering Scald Awareness Task Group, and other safety-promotion organizations recommend that home hot-water heater thermostats be set at 49°C to 53°C (120°F to 130°F) to reduce scalding risks (Lukefar and Ezekial, 1994)[4]. CDC (2009) recommends that hot-water heaters be kept below 49°C (120°F) to minimize the risk for scalding in the home. Most municipalities and state regulations recommend that home hot-water heater temperatures remain below 49°C (120°F), since most burns occur in the home and not at hospitals

[4] See http://www.asse-plumbing.org/WaterHeaterScaldHazards.pdf.

or rehabilitation facilities where there are more at-risk patients (CDC, 2009; Haik, 2007; Tung et al., 2005). Maximum allowable temperatures in hospitals and healthcare organizations are often regulated by states. Data from 39 states reported regulating maximum allowable hospital water temperature from as low as 43°C (110°F) to as high as 53°C (130°F) (Mandel et al., 1993).

Table 4-3 shows the trade-off between scald risk and the risk of *Legionella* growth. This table was submitted for inclusion in the 2020 Uniform Plumbing Code pending a member vote. In buildings with sensitive populations, the production and storage of hot water at greater than 60°C (140°F) will likely require the use of thermostatic mixing valves to blend cold and hot water to appropriate temperatures at the tap. It is important for these devices to be routinely serviced and for temperature to be monitored closely (Bédard et al., 2015; Johansson et al., 2006).

TABLE 4-3 Water Temperature, Risk of Scalding/Burning, and *Legionella* Growth Potential

°F	°C	Time to First-degree Burn	Time to Second-degree Burn	*Legionella* Growth Potential
<77	<25			No
80	27			Low
90	32			Moderate
100	38			Very high
110	43			Very high
116	47	35 min	45 min	Moderate
122	50	1 min	5 min	Very low
131	55	5 sec	25 sec	No
140	60	2 sec	5 sec	No
149	65	1 sec	2 sec	No
154	68	instantaneous	1 sec	No

SOURCE: Adapted from Armstrong (1978) and Klein (2018).

Disinfection

Maintenance of a disinfectant residual can be an integral part of a building's water management plan for control of *Legionella*. Disinfection methods should be paired with scheduled water testing to ensure that the system maintains a residual. Many of the disinfectants reviewed below have demonstrated at least some degree of efficacy towards management of *Legionella* in drinking water distribution systems and building water systems. Hence, the choice, and success, of disinfection technology will depend on additional considerations such as cost, operator training, materials (corrosion), water chemistry, system configuration, and water use patterns.

Chemical Disinfection

Chemical disinfectants, particularly oxidizing agents such as chlorine, chlorine dioxide, chloramine, and ozone, are widely used to control *Legionella* spp. and protozoa—both as disinfectants in

drinking water distribution systems and as secondary disinfectants within buildings. The disinfectant should ideally inactivate microorganisms in the bulk water, but also penetrate and inactivate microorganisms associated with biofilms. Overall, the efficacy of disinfectants depends on the culture condition of *Legionella* spp. and their host protozoa and the physicochemical characteristics of the water (e.g., temperature, pH, organic carbon, hardness).

Disinfection strategies are sometimes evaluated in terms of "CT" or disinfectant concentration (measured in mg/L) multiplied by time of exposure (measured in minutes). Very high disinfectant levels (4 mg/L or more) applied for many hours might be recommended when responding to an outbreak in a hospital or nursing home but would be impractical and excessive for routine water treatment in premise plumbing. Choice of a disinfectant also needs to consider corrosion impacts on pipe materials, reliability, and safety. Because *Legionella* spp. can use protozoa and their cysts as a protective shield against disinfectants, it is imperative to consider the efficacy of each disinfectant for both organisms. In some systems, multiple points of application are necessary to maintain chemical residuals throughout the entire network.

Chlorine. Chlorine is the most commonly used disinfectant by water utilities in the United States. Chlorine adversely affects the cell membrane, nucleic acids, respiration, and enzymatic activity of microbes, leading to their inactivation (Kim et al., 2002). During treatment, chlorine can be added to water as elemental chlorine (chlorine gas), sodium hypochlorite solution, or dry calcium hypochlorite. In water, chlorine exists as hypochlorous acid and hypochlorite ion, where the hypochlorous acid predominates when pH is below 7.5 and is a more effective biocide.

Generally, maintenance of a free chlorine residual in potable water systems is effective for control of *Legionella* spp. (Kim et al., 2002). For example, planktonic *Legionella* spp. resuspended in water were eliminated within three minutes by 2 mg/L free chlorine derived from sodium hypochlorite (Miyamoto et al., 2000). Mouchtouri at al. (2010) disinfected *Legionella*-positive cooling towers by circulating water with 5 mg free chlorine/L for five hours. Systems with pH greater than 8.0 received higher free chlorine dosages of 15 to 20 mg/L to achieve the required disinfection level; disinfection was considered successful when samples showed concentrations less than 1 CFU/mL (10^3 CFU/L). Hyperchlorination with 4 to 6 mg/L decreased *L. pneumophila* in plumbing systems by 5 to 6 logs over six hours (Muraca et al., 1987). The decline in *L. pneumophila* was more rapid at 43°C than at 25°C. However, a higher dose of chlorine was required at 43°C to overcome thermal decomposition and maintain a chlorine residual of 4 to 6 mg/L. The high temperatures likely accelerated chlorine reactions with demand-causing compounds, including natural organic matter and reduced metals like iron or manganese.

The ecology of *Legionella* plays an important role in disinfection efficacy; whether the bacteria is shielded from the disinfectant depends on whether it is planktonic or within a protozoan trophozoite or cyst. Amoebae cysts are much more resistant to disinfection than the free-living trophozoite (De Jonckheere and Van de Voorde, 1976). *Legionella* spp. in protozoa cysts survived 25-fold more chlorine disinfectant than planktonic cells after 18 hours (Kilvington and Price, 1990). Dupuy et al. (2011) showed that co-culture significantly increased survival of *L. pneumophila* at 30°C, but not at 50°C.

Guidelines for the maintenance of continuous chlorine residuals in building premise plumbing to prevent amplification of *Legionella* tend to recommend residual concentrations similar to those required in drinking water distribution systems. The Allegheny County (Pennsylvania) Health Department specifies that potable water, from entering a building through to all outlets (e.g., faucets, showerheads), should maintain at least 0.3 mg/L free residual chlorine (Moore and Shelton, 2014). The California Code of Regulations, Title 22, Section 60306, requires that industrial or commercial cooling towers maintain a 0.3 to 0.7 mg/L free chlorine residual (State of California Energy Commission Staff, 2004).

Chlorination can have adverse effects on the plumbing system by making the water acidic, which in turn can make the water more corrosive to pipes, joints, fittings, and fixtures. If chemical flushing is used with hyperchlorination, these adverse effects can be more pronounced.

Chlorine Dioxide. Unlike free chlorine, chlorine dioxide does not hydrolyze when it enters water; it remains a dissolved gas in solution. As a neutral compound, it can easily diffuse through cell membranes of microorganisms where it disrupts protein synthesis. It is typically generated on site for immediate use by slowly adding a strong acid (e.g., hypochlorous or sulfuric acid) to a sodium chloride solution.

Chlorine dioxide has been found to be more effective in penetrating biofilms than chlorine (Kim et al., 2002; Lin et al., 2011; Walker et al., 1995), and it is effective over a wider pH range (Lin et al., 2011). Loret et al. (2005) evaluated 0.5 mg/L chlorine dioxide for control of *Legionella* grown in biofilms in a pilot-scale premise plumbing system incubated at 30°C. *Legionella* populations decreased to undetected levels (less than 500 CFU/L) within six days of treatment. As with chlorine, the presence of amoebae reduces the efficacy of chlorine dioxide disinfection of *Legionella* (Dupuy et al., 2011). Despite the effectiveness of chlorine dioxide, it is not commonly used as a disinfectant in the distribution system due to the toxicity of the disinfectant and some of its byproducts (EPA, 1998) and the potential for objectionable odors (Dietrich et al., 1991).

There have been a handful of real-world applications of chlorine dioxide treatment of premise plumbing. Walker et al. (1995) reported elimination of *Legionella* spp. to below detection in a hospital water system after treatment with 50 to 80 mg/L chlorine dioxide. Srinivasan et al. (2003) evaluated the use of chlorine dioxide (0.3 to 0.5 mg/L residual) for 17 months in a hospital and found *Legionella* occurrence decreased from 41 percent to 4 percent in distal sites. Only *L. anisa* was recovered during the chlorine dioxide treatment and it was cultured from both the hot- and the cold-water systems. No cases of nosocomial *Legionella* infection were detected in the building with the chlorine dioxide system during the 17-month evaluation. Marchesi et al. (2013) reported reduction in *L. pneumophila* contamination in three hospital hot-water (60°C) systems over a three-year period using a chlorine dioxide dose of 0.50 to 0.70 mg/L and a targeted residual of 0.3 mg/L at distal sites. Cristino et al. (2012) described use of chlorine dioxide after shock treatment to maintain 0.3 mg/L residual at the tap after 5 minute of flushing in a hospital. *Legionella* counts remained acceptable (less than 10^3 CFU/L), and no cases of hospital-acquired legionellosis occurred during the study period. Zhang et al. (2009) reported that after installation of a chlorine dioxide system it took months to achieve a 0.11 mg/L chlorine dioxide residual within two hospital systems, but the occurrence of *Legionella* at hot-water taps decreased from 60 percent to less than 10 percent of sampling sites, and no cases of hospital-acquired Legionnaires' disease were detected.

Monochloramine. Monochloramine is formed by adding free chlorine in a solution of ammonium chloride at a chlorine-to-nitrogen molar ratio of 0.5 (pH 8.5). Disinfection with monochloramine has gained traction in the United States because the disinfectant is more stable in the distribution system, it minimizes the formation of disinfection byproducts, and it can penetrate biofilms better than free chlorine (LeChevallier et al., 1988; Lee et al., 2011; Pressman et al., 2012). Monochloramine has a lower chlorinous odor threshold than free chlorine (EPA, 1994), but it has a much lower disinfection efficacy than free chlorine (Symons, 1978) and requires a much longer contact time or higher dose if used as a primary disinfectant.

One of the challenges with using monochloramine, particularly within a building system, is properly managing the chlorine-to-ammonia ratio (4.5:1) at an optimum pH (8.3) in order to form monochloramine without stimulating nitrification within biofilms. Nitrification is a microbial growth process by which ammonia is sequentially oxidized to nitrite and nitrate. Nitrite catalyzes the decay of chloramines

and can leave a system without disinfectant residual and hence even more vulnerable to bacterial regrowth. Nitrifying bacteria fare better at warmer temperatures, making nitrification a summer problem for water utilities, which often implement flushing campaigns and even temporarily convert to free chlorine. Nitrification can be even more problematic in buildings because some premise plumbing is consistently maintained at a warm temperature, there is a high surface area-to-volume ratio for biofilm formation, and stagnant conditions can be especially conducive to slow-growing autotrophic organisms like nitrifiers and stimulate further decay of chloramines (Zhang and Edwards, 2009)—all of which could potentially undermine chloramine disinfection systems in premise plumbing.

As a disinfectant in the water supply distribution system, chloramines appear to be more effective than free chlorine in reducing the overall risks from *Legionella*. Kool et al. (1999) examined 32 hospital-acquired (nosocomial) outbreaks of Legionnaires' disease from 1979 to 1997 where drinking water was implicated. They found that the odds of a nosocomial *Legionella* outbreak were 10.2 times higher in hospitals supplied by a water system that maintained free chlorine than in those supplied by a water system using a chloramine residual. Similar results were obtained by Heffelfinger et al. (2003), who surveyed 152 hospitals with reported cases of hospital-acquired Legionnaires' disease. Flannery et al. (2006) showed significant reductions in the occurrence of both amoeba and *Legionella* spp. in building plumbing systems in San Francisco after the utility converted from free chlorine to chloramines. The prevalence of amoebae decreased from 169 of 1,405 (12 percent) samples when chlorine was used to 78 of 944 (8 percent) samples collected after conversion to monochloramine. Prior to the conversion, *Legionella* spp. were cultured from 61 of 169 (36 percent) samples in which amoebae were present versus 291 of 1,236 (24 percent) samples without amoebae. After conversion to monochloramine, *Legionella* were found in 1 of 78 (1 percent) samples containing amoebae and 8 of 866 (1 percent) samples without amoebae. *Legionella* occurrence was also reduced in 96 buildings in Pinellas County, Florida, when the drinking water distribution system converted from chlorine to monochloramine disinfection (Moore et al., 2006). When free chlorine was used, 20 percent of the buildings were colonized with *Legionella* in at least one sampling site. Within a month after chloramination, *Legionella* colonization was reduced by 69 percent. Monochloramine appeared to be more effective in reducing *Legionella* in hotels and single-family homes than in county government buildings, perhaps because of more consistent water usage.

Chloramines also appear to be more effective than chlorine when used as a treatment in buildings. Coniglio et al. (2015) studied the addition of monochloramine after two hospital hot-water systems failed to control *Legionella* with thermal treatment (65°C to 70°C), shock chlorination (50 mg/L free chlorine for one hour at distal sites), point-of-use filters (0.2 micron), and hydrogen peroxide (17 mg/L). Prior to chloramine treatment, 100 percent of samples were positive with *L. pneumophila* serogroups 3 and 6. Monochloramine treatment began at 3.0 mg/L and was then reduced to 2.0 to 2.5 mg/L after one month. *Legionella* was not detected during the following year except for one month when the monochloramine generator failed for 15 days. In a three-year study of monochloramine addition to a hospital in Italy, Marchesi et al. (2012, 2013) reported that a residual between 1.5 and 3.0 mg/L effectively controlled *Legionella* occurrence, with seven of the eight positive samples occurring within the first eight months and the eighth positive sample occurring at 15 months, when the monochloramine dose decreased below 1 mg/L.

Not all studies have been as straightforward, however. Duda et al. (2014) showed that although monochloramine concentrations of 1 to 4 mg/L significantly reduced the occurrence of *Legionella* in a hospital hot-water system (with the average number of positive sites declining from 53 percent to 9 percent), during certain months when nitrate, total ammonia, and pH levels were elevated, the percentage of positive samples increased, suggesting inadequate control of the chloramination process and nitrification. *Legionella* speciation changed from 90 percent of samples testing for *L. pneumophila* serogroup 1 to only 49 percent post-disinfection, while *L. bozemanii* occurrence increased.

The effectiveness of monochloramine is generally thought to be due to its ability to penetrate biofilms and inactivate the bacteria (Donlan et al., 2002; LeChevallier et al., 1988). Lee et al. (2011) and Pressman et al. (2012) both used microelectrodes to demonstrate that monochloramine had greater penetration into biofilms than chlorine, but this penetration did not necessarily translate to immediate loss of viability. Johnson et al. (2018) found that amoebae in five free chlorinated reclaimed water systems were mostly (50 percent to 95 percent) in the active trophozoite phase; however, in the chloraminated system, 87 percent of the mesophilic amoebae and 66 percent of the thermophilic amoebae were in the cyst phase. They hypothesized that the penetration of chloramines into the biofilm might trigger the amoebae to form cysts rather than outright kill the protozoa. Since *L. pneumophila* only amplifies in the trophozoite stage, it may be possible to manage *Legionella* risk by limiting the free-living trophozoite population. Additional research is needed to examine the precise action of monochloramine on *Legionella* persistence and growth within pipeline biofilms.

Ozone. Ozone attacks unsaturated bonds of aldehydes, ketones, and carbonyl compounds (Langlais et al., 1991) and can participate in electrophilic reactions with aromatic compounds and neutrophilic reactions with many cellular components (i.e., fatty acids, carbohydrates, amino acids, proteins, nucleic acids). These reactions collectively affect the cytoplasmic membrane of bacterial cells and their protein structure as well as DNA. However, because ozone does not form a stable residual and decomposes rapidly in water, it is not typically used for building plumbing systems, but primarily to disinfect water supplies.

Several laboratory studies have evaluated ozone for inactivating *Legionella* (Domingue et al., 1988; Muraca et al. 1987) and amoebae cysts (Langlais and Perrine, 1986; Wickramanayake et al., 1984). There are few studies of using ozone to treat a building water system. Edelstein et al. (1982) applied continuous ozonation to the water of one wing of an unoccupied hospital building while the other wing used chlorinated tap water. The results were inconclusive, with both the ozonated and chlorinated sections having some positive results for *Legionella* (three of 12 samples positive for the ozone treatment, eight of 12 samples positive for the chlorine treated wings). Moreover, when the ozone was discontinued *L. pneumophila* regrew and reached levels similar to the pre-treatment densities. The authors noted that residual ozone at a faucet or shower would be released as a gas and could create a health hazard if inhaled.

Ultraviolet Irradiation

Ultraviolet (UV) light may not directly kill microorganisms but rather damages their DNA and proteins, which prevents them from replicating and becoming infectious. UV intensity times the duration of exposure is commonly referred to as fluence (mJ/cm^2) and describes UV disinfection capability. Fluence represents the energy per unit area falling onto a surface. Maximum efficacy with UV is attained at 254 nm (Kim et al., 2002) but turbidity, natural organic matter content, and particulate matter can affect UV disinfection capability. Medium-pressure UV light sources may also generate higher wavelength UV light (268 and 286 nm) that impacts proteins more than nucleic acids (Beck et al., 2017). Because UV does not provide a residual, it is only effective at the point of treatment and is typically combined with a chemical disinfectant for distributed water to effectively control *Legionella* spp.

All *Legionella* isolates tested by Cervero-Aragó et al. (2014) required 5 to 6 mJ/cm^2 UV fluence to inactivate 4 logs. However, a higher fluence was required when *Legionella* was co-cultured with amoeba. Muraca et al. (1987) found that UV irradiation at 30 mJ/cm^2 reduced *L. pneumophila* by 5 log units in 20 minutes although the very high concentrations of the bacteria could have affected the UV adsorption of the suspension. *Legionella* inactivation requires slightly higher doses when the bacteria are exposed

to light repair (i.e., DNA repair mediated by enzymes activated by visible light), but has a similar level of inactivation when either low-pressure or medium-pressure lamps are used (see Table 4-4). Notably, when amoeba co-culture was used on samples below detection using buffered charcoal yeast extract (BCYE) agar plates, VBNC-like cells were resuscitated (Grossi et al., 2018). Hence, previous reports only using plate culture to assay inactivation may overestimate actual UV inactivation, particularly for higher wavelength UV light.

Hijnen et al. (2006) reported a log reduction of *Acanthamoeba* spp. with 40 mJ/cm^2. A 3-log inactivation of various *Acanthamoeba* species and *Vermamoeba vermiformis* was achieved with fluences of 23 to 100 mJ/cm^2; the higher levels were required for cyst inactivation. Overall, inactivation of *Acanthamoeba* spp. and *V. vermiformis* required higher levels of UV compared to *Giardia* or *Cryptosporidium* (EPA, 2006).

TABLE 4-4 UV Doses (mJ/cm^2) for Inactivation of *L. pneumophila*

L. pneumophila Strain	Lamp Type	1-log	2-log	3-log	4-log
Philadelphia Type 2	LP	0.92	1.84	2.76	No data
Philadelphia 1 (no light repair)	LP	0.5	1	1.6	No data
Philadelphia 1 (with light repair)	LP	2.3	3.5	4.6	No data
Philadelphia 1 ATCC33152	LP	1.6	3.2	4.8	6.5
Philadelphia 1 ATCC33152	MP	1.9	3.8	5.8	7.7

NOTES: LP = low-pressure lamps, which have a single output around 254 nm. MP = medium-pressure lamps, which have polychromatic output at multiple wavelengths. SOURCES: EPA (2016); Knudson (1985); Oguma et al. (2004).

Copper-Silver Ionization

The use of copper-silver (Cu-Ag) ionization to control *Legionella* in building water systems is widespread, partly because it is relatively low cost and low maintenance compared to other controls. Copper (Cu) and silver (Ag) both have biocidal activity, especially when used in combination. In ionization chambers, both metals can be ionized through electrolysis to form positively charged ions. The copper ions interact with negatively charged cell walls of *Legionella* spp. (and other bacteria), disrupting cell wall permeability and subsequent nutrient uptake. The copper ions penetrate the cell wall and create an entrance for silver ions, which bond with DNA, RNA, cellular proteins and respiratory enzymes, immobilizing the cell and curtailing cell division.

Field studies constitute the majority of the published reports on the efficacy of copper-silver ionization for controlling *Legionella* in building plumbing systems (Blanc et al., 2005; Chen et al., 2008; Demirjian et al., 2015; Dziewulski et al., 2015; Kusnetsov et al., 2001; Liu et al., 1994, 1998; Mòdol et al., 2007; Rohr et al., 1999; States et al., 1998; Stout and Yu, 2003). These reports typically describe applying copper-silver ionization to remediate situations where *Legionella* have already colonized the system. Most studies have looked at the disinfection effects of these ions used together, but Lin et al. (1996) examined the effects of each ion individually. They reported 6-log reduction of *L. pneumophila* serogroup 1 in 2.5 hours with 0.1 mg/L copper. Similarly, a 6-log reduction *L. pneumophila* was obtained within six hours on exposure to a solution of 50 µg/L silver ions (Miyamoto et al., 2000). Cloutman-Green et al. (2019) reported effective *Legionella* management in a healthcare building hot-water system operated at 42°C (range 37°C to 44°C) supplemented with copper-silver ionization operated at 0.37/0.034 mg/L,

respectively. The authors reported a reduction in energy and carbon emissions of 33 percent and 24 percent, respectively, compared to an equivalent temperature-controlled system.

June and Dziewulski (2018) provide an excellent review of copper-silver ionization for the inactivation of *Legionella.* The review suggests that there have been mixed results when considering the efficacy and reliability of copper-silver ionization for controlling *Legionella*. Copper-silver ionization is slower acting compared to other disinfectants and more dependent on water chemistry (e.g., pH, total dissolved solids or TDS), as the silver can precipitate in the presence of high dissolved solid concentrations, becoming unavailable for disinfection. *Legionella* can be protected from copper and silver ions when associated with biofilms or amoebae, and the potential for *Legionella* to develop resistance to copper and silver ions has been suggested (EPA, 2016a). Indeed, dominant sequence types of *L. pneumophila* isolated from two hospitals' hot-water systems with and without copper-silver ionization have been shown to be highly resistant to copper (Prévost et al., 2017). The development of resistance to copper and silver may be a concern in ensuring the long-term efficacy of copper-silver ionization. Longitudinal case studies report that copper-silver ionization can become ineffective for the control of *Legionella* in biofilms and water in large existing healthcare facilities (Blanc et al., 2005; Rohr et al., 1999). A further concern is that bacteria that develop resistance to heavy metals may also develop antibiotic resistance (Chen et al., 2015), although additional research is needed to determine if there is an increase in antibiotic resistance in water treated with copper-silver ionization. June and Dziewulski (2018) suggest approaches for improving copper-silver ionization efficacy and reliability, including increasing the dissolved oxygen and sodium content of the treated water, applying copper and silver ions in combination with other disinfectants, and using copper and silver ions at higher temperatures.

Other Disinfecting Agents

Bromine behaves similarly to chlorine, existing in water as hypobromous acid to form HOBr and OBr^- depending on the pH (Kim et al., 2002). Bromine has generally less efficacy against *Legionella* spp. compared to chlorine. Bromine, iodine, and iodophore are variously effective against *Acanthamoeba culbertsoni* and *Naegleria fowleri* cysts (De Jonckheere and Van de Voorde, 1976). Although used for potable water disinfection in some emergency instances, use of bromine, iodine, or hydrogen peroxide in water supply distribution systems and building water systems is not widely practiced.

Peracetic acid is thought to disinfect by impacting lipoproteins in the cell membrane (Rossoni and Gaylarde, 2000). Unlike chlorine and hydrogen peroxide, its potency is not greatly compromised by organic matter or enzymes (Baldry et al., 1991), and it has acceptable potency at neutral pH and can be effective for biofilms (Rossoni and Gaylarde, 2000). However, peracetic acid has had limited use within building plumbing systems.

Non-oxidizing biocides such as BNPD (2-bromo-2-nitropropane-1, 3-diol), glutaraldehyde, guanidines, dithiocarbamates, isothiazolin, halogenated amides such as DBNPA (di-bromo-nitrilo-propionamide), halogenated glycols such as bronopol (2-bromo-2-nitroproprionamide), and some quaternary ammonium compounds are commonly used in cooling towers (Kim et al., 2002). Among non-oxidizing biocides, glutaraldehyde, DBNPA, isothialozin and bromopol were found to be effective against to varying degrees (Kim et al., 2002). The biocides MBC-115 [a quaternary ammonium comprised of poly(oxyethylene (dimethyliminio) ethylene (dimethyliminio) ethylene dichloride)] and MBC-215 (an isothiazine derivative of a mixture of 5-chloro-2-methyl-4-isothiazolon-3-one and 2-methyl-4-isothiazolin) have been widely used in cooling towers to control *Legionella* spp. Berk et al. (1998) found the efficacy of both

compounds on *Legionella* spp. to be poor, although this may have been due to the presence of amoebae. Barker et al. (1993) found that the antiseptics polyhexamethylene bioguanide and benzisothiazolone were ineffective against *L. pneumophila* grown with *A. polyphaga* compared to *L. pneumophila* pure cultures. Both biocides attack the bacteria cell membrane; amoebae proteins coating *Legionella* may have conferred biocide resistance. Miller and Simpson (1999) reaffirmed the resistant nature of protozoa cysts to disinfection with some of these alternative compounds.

Manage Hydraulics

Appropriate hydraulic system design and maintenance are essential for effective *Legionella* control. In particular, hydraulics are essential to maintaining and delivering water at an inhibitory temperature as well as distributing disinfectants throughout the building. Recent guidelines following years of mandatory *Legionella* control in Europe stress the need to properly manage hydraulics to ensure homogeneous temperature and biocidal control in all areas of the hot-water system, including balancing under varying demand (Centre Scientifique et Technique du Bâtiment, 2012; Health and Safety Executive, 2013). Construction and operational standards for buildings often specify minimizing stagnation (e.g., via recirculation loops, elimination of hydraulic and physical dead ends).

In many cases, differences among reports on the efficacy of thermal control on *Legionella* probably reflect whether the temperature set points were hydraulically achieved across the whole system, including at the outlets (faucets and showers). For example, a single piece of deficient equipment such as backflow preventers on a single mixing valve can influence the hot-water temperature distribution within an entire building wing, causing hot-water temperature to decrease in those sectors (Boppe et al., 2016). The presence of stagnation caused by dead legs, inadequate system hydraulic balancing, or lack of occupancy also reduces the disinfectant efficiency in these areas. As a global recommendation, extended periods of stagnation and the presence of dead legs should be avoided. To reach this goal, minimum water velocity should be maintained at all times within the recirculation pipes. The Centre Scientifique et Technique du Bâtiment (CSTB, 2012) proposes maintaining the highest value between 0.2 m/s and the velocity required to maintain heat loss below 5°C.

Flushing to Control Distal Growth

Flushing of water can have significant benefits in terms of water quality and more specifically *Legionella* levels. Flushing can reduce total cell counts in premise plumbing by dislodging loose deposits and biofilm, which tend to harbor higher levels of heavy metals, *Aeromonas*, ATP (indicator of biological activity), and *Legionella* as judged by operational taxonomic units quantified by amplicon sequencing (Liu et al., 2017). Flushing systematically reduces total and viable bacterial cells and heterotrophic plate counts in large buildings (Bédard et al., 2018; Lautenschlager et al., 2010), and in most instances will lower the concentrations of *L. pneumophila* concentrations in household and hospital taps (Bédard et al., 2019; Cristina et al., 2014). Lipphaus et al. (2014) found that flushing reduced total cell counts by flow cytometry in infrequently used cold-water hospital taps, but had a less pronounced effect on hot-water taps. Periodical flushing of water is particularly useful to prevent colonization and limit the growth of *Legionella* at the distal sites of cold- and hot-water systems. Manual flushing is recommended in guidance and is widely used during building commissioning or after periods of vacancy (e.g., weekends, vacations).

There is no consensus on the optimal flushing frequency to prevent *Legionella*. Several guidance documents recommend weekly flushing of low-use faucets and showers (e.g., ECDC, 2017; HSE, 2013). A

much higher flushing frequency was suggested by Totaro et al. (2018)—a study done in an Italian hospital that was experiencing elevated *L. pneumophila* positivity and concentrations, despite optimal temperature control and on-site addition of chlorine dioxide. Five dead-end locations and the main return loop were all positive for *L. pneumophila* serogroups 3 and 10–14 (concentrations ranging from 8 x 10^3 to 1.3 x 10^5 CFU/L) before the installation of time-flow taps. Operating the five time-flow taps for one minute every six hours (64 L per day) slightly decreased the *Legionella* concentrations. After further increasing the flushing frequency to one minute every two hours (192 L per day), no positives were observed. These findings suggest that implementing automated periodic flushing may be necessary if hydraulic corrective actions such as the elimination of dead legs and the balancing of flows cannot be implemented.

Storage facilities and dead-end pipes where water velocities and turnover can be very low are locations that are more susceptible to biofilm development. Sediments can accumulate in areas of low flow, increasing disinfection demand and promoting bacterial growth. Stratification caused by warm water temperatures can prevent adequate mixing. Inlet–outlet configurations can result in "last in, first out" flow patterns in which older water never leaves the storage tank, causing stagnation, dissipation of disinfectant residuals, and microbial growth. Increasing the frequency of storage tank cleaning will minimize sediment accumulation and help control biofilms.

Relationship Between Flow Rates and Biofilm Formation in Pipes

Higher flow rates and turbulence can reduce biofilm formation (Donlan et al., 1994; Kirisits et al., 2007). At lower residence time, the erosion of cells on the surface due to higher shear force and enhanced diffusion of disinfectant within a thinner boundary layer are factors suggested to explain the effect of flow dynamics on biofilm formation (Donlan et al., 1994). A study in which biofilms were first established under laminar or turbulent flow looked at the effect of unsteady hydraulic conditions on the biological quality of the drinking water (Manuel et al., 2010). Once the biofilm was established, periods of stagnation promoted bacterial accumulation for both the planktonic and biofilm bacteria. These cells were carried away once the flow was resumed, increasing the bacterial concentration in drinking water. Similarly, the ratio of *L. pneumophila* cell detachment from biofilm following exposure to 0.1, 0.3, and 0.7 m/s was found to increase with flow velocity (Shen et al., 2015). Initial adherence of *L. pneumophila* strains to an existing biofilm was conducted in quasi-stagnant conditions (0.007 m/s) prior to exposure to water flow. The same trends were observed both in smooth and rough biofilm, although *L. pneumophila* adhesion was enhanced by biofilm roughness. This enrichment was attributed to increased interception of the suspended *L. pneumophila* in flowing water on biofilm surface (Shen et al., 2015).

Dissimilar results have been found by others. The impact of turbulent, transition and laminar flow on existing and newly formed biofilm was investigated by Tsagkari and Sloan (2018). They found that turbulent flow did not reduce biofilms; instead, biofilm thickness and density increased under turbulent flow conditions equivalent to 0.25 m/s in a 30.3-mm diameter pipe. Another key parameter is the surface-to-volume (S/V) ratio, which fundamentally drives the relative amount of surface area available to colonize and overall biomass production potential for pipes (Tsvetanova and Hoekstra, 2012). The authors observed a significant effect of S/V ratio on the planktonic biomass, with concentrations 4 to 14 times higher with higher S/V ratios. Premise plumbing piping usually has a small diameter and thus a larger S/V ratio than the distribution system.

There are few methodologies available to assess, in detail, hydraulically deficient areas within an existing water system. CSTB (2012) suggests investigating common causes such as valve obstructions

(leading to stagnation or reduced water velocity within the return loop), type of control elements installed, re-circulation pump design and operation, and the lack of balance between the different secondary flow and return loops. Given the intimate relationship between temperature and hydraulics, temperature is not only a very effective proxy for residence time, but also relatively easy and inexpensive to monitor (Bédard et al., 2015). Systems that fail to maintain control temperatures at the point of use despite adequate water heater temperatures are considered at risk and hydraulically deficient.

Nutrient Control

An indirect strategy for management of *Legionella* in building water systems could be controlling biofilms, which are the food source for free-living protozoa (Characklis and Marshall, 1990; LeChevallier et al., 2011; NRC, 2006). One of the most common ways to control biofilms is to limit nutrients in the water—a strategy used by some western European countries that also tend to distribute potable water with little or no disinfectant residual (Bartels, 2018; Exner, 2018). Hence, much of the work investigating the effect of limiting organic carbon on biofilm growth, and hence on *Legionella,* has been conducted in The Netherlands.

A substantial portion of the organic carbon present in drinking water is derived from complex natural organic matter (for example, from decaying leaves), a form that cannot be directly utilized by microorganisms. Thus, a direct measurement of total organic carbon does not indicate the fraction that is actually bioavailable to drinking water microbes. Instead, bioassays have been developed to directly measure the biodegradable fraction of organic carbon in the water, specifically the assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC) assays. Organic carbon levels in U.S. drinking water supplies typically average 100 μg/L for AOC (ranging from 50 to 250 μg/L) and 0.3 mg/L for BDOC (ranging from 0 to 1.0 mg/L); surface water supplies have higher levels of biodegradable organic matter than groundwater supplies (LeChevallier et al., 1996; Volk and LeChevallier, 2000).

In terms of setting nutrient limits for water exiting a drinking water treatment plant, only extremely low levels of AOC (less than 50 μg/L) have been observed to have a measurable effect on downstream numbers of total bacteria as judged by heterotrophic plate counts (HPCs) or ATP (LeChevallier et al., 1991). Much lower AOC levels of 5 to 10 μg/L were associated with lower *L. pneumophila* levels in Dutch drinking water distribution systems (van der Kooij and van der Wielen, 2014). The same research group also observed a strong correlation among AOC, biofilm concentration, and *L. pneumophila* growth, with no growth observed at AOC levels below 1 μg/L (van der Kooij et al., 2017). Similarly, Learbuch et al. (2019) treated water with a pilot reverse-osmosis system and subsequent remineralisation to obtain very low AOC levels and showed that the water did not support growth of *L. pneumophia*. On the other hand, Williams et al. (2015) performed extensive bench-scale tests in simulated glass water heaters with spiked AOC levels ranging from 0 to 15,000 μg/L over 17 months and could find no correlation with *Legionella* concentration, although total bacterial numbers by HPCs did correlate.

It is important to recognize that such low AOC levels can be very difficult to achieve and maintain in drinking water because AOC can be generated in water mains and by the bacteria native to the plumbing. Dai et al. (2018) conducted a bench-scale study of controlled, replicated simulated glass water heaters representing a range of premise plumbing conditions that were fed biofiltered water (to simulate the AOC removal process used at water treatment plants or in whole-house filters). Although biofiltering the water substantially reduced the TOC and 16S rRNA gene copy numbers, there was no measurable effect on *Legionella* gene copy numbers. Instead, the individual plumbing conditions, such as the presence of iron corrosion sediments, nitrification, or cross-linked polyethylene (PEX) pipe material leaching organic carbon, dominated the effects on the microbial community composition and, in some cases, *Legionella*.

Iron Corrosion and Inorganic Nutrients

Much of U.S. water distribution systems consist of century-old unlined iron mains, which are beyond their designed lifespan and subject to substantial corrosion as well as intrusion during water main breaks. Corrosion of pipe surfaces provides not only a habitat for bacterial proliferation and protection from chlorine disinfectant residuals but also a source of nutrients. Aerobic microbial respiration consumes oxygen, resulting in a reduced redox environment that can accelerate corrosion and produce a disinfectant demand. Corrosion of pipe surfaces and deposition of corrosion products can also create tubercles and surface roughness that protect biofilm organisms from hydraulic shear (Characklis and Marshall, 1990). The resulting turbulent flow can help transport nutrients and detritus, further enhancing the biofilm environment.

Growth of certain microbes is also promoted by other inorganic substances can also serve as electron donors or acceptors including methane, ferrous iron, reduced sulfur compounds, hydrogen gas, manganese, ammonia, and nitrite. These substances can stimulate autotrophs to fix organic carbon into the system, leading to more bacterial cells and associated organic matter. The accumulation of organic carbon and reduced inorganic compounds (e.g., iron, nitrite, sulfides) in biofilms can create a disinfectant demand that protects the attached microbes from being inactivated. In particular, iron-oxidizing bacteria oxidize ferrous iron to produce ferric iron oxides. Not only is iron a known nutrient for *Legionella*, it also reacts with chlorine, thereby increasing microbial risk by removing the disinfectant residual.

Plumbing Materials

Plumbing materials are an important factor to consider in *Legionella* control. Common plumbing materials in buildings include copper, iron, and numerous plastics, with cross-linked polyethylene (PEX) and cross-linked polyvinyl chloride (PVC) being particularly suitable for hot-water plumbing because of their tolerance of higher temperatures. Each pipe material will influence the building-level water chemistry and shape the biofilms that colonize premise plumbing in a unique manner (Ji et al., 2015). Being able to identify a pipe material that most effectively limits proliferation of *Legionella* for a given water chemistry and building type would be valuable as a passive barrier. It is important to recognize that water chemistry varies regionally, seasonally, and as dictated by various upstream water treatment processes (Dai et al., 2018), making it difficult to predict how incoming water will react with different pipe materials.

Although copper pipe has well-known antimicrobial properties, it does not universally control *Legionella.* Indeed, copper has been associated with decreased, increased, and comparable numbers of *Legionella* relative to other pipe materials (Rhoads et al., 2017b). As described in Chapter 2, the age of copper pipe, temperature, pH, and general water chemistry influence the dissolution chemistry and overall antimicrobial action of copper towards *Legionella*. The composition of the biofilm community also matters, e.g., interactive effects of amoebae and copper appear to favor survival of *Legionella* (Buse et al., 2017; Ji et al., 2017). Thus, it is clear that copper pipe cannot be the sole agent to control *Legionella*; other microbiological, chemical, and site-specific factors needs to be considered.

PEX and other heat-tolerant flexible polymeric plastic materials have gained popularity for their ease of use for hot-water plumbing. These materials, however, are well known to leach organic carbon and can stimulate bacterial growth (Proctor et al., 2018). In particular, flexible pipe materials commonly employed to plumb showerheads are especially vulnerable to biofilm formation and microbial growth, producing total bacterial cell counts ranging from 10^6 (PE-Xc—applied as a rigid control plastic) to 10^8

(PVC-P) cells/cm^2 of hose (Proctor et al., 2016). A comprehensive comparison of six different shower pipe materials indicated that these materials had a profound influence on the microbial community composition, including the occurrence of genera containing *Legionella* and other pathogens (Proctor et al., 2016). However, interestingly, *Legionella* operational taxonomic unit were lower when total bacterial cell counts were higher, suggesting *Legionella* were out-competed. An eradication strategy based on this probiotic concept is discussed later in this chapter.

Iron pipe is extremely vulnerable to biofilm formation, partly because of its susceptibility to corrosion. Even without corrosion and with depleted AOC and sufficient chlorine residual, iron is highly prone to biofilm build-up compared to other materials, such as PVC (Camper, 1996). Iron pipes also support a more diverse microbial population than do PVC pipes (Dai et al., 2018; Norton and LeChevallier, 2000). While no longer used in modern buildings, legacy iron pipe remains common in older buildings, water mains, and service lines. One major survey found that cast iron pipes comprise an estimated 38 percent of water distribution system pipes in the United States (McNeill and Edwards, 2001). Even in modern systems built without iron, other sources, such as steel components in water heaters, can elevate iron levels in water. When iron components corrode, they not only release iron into the water, but in the process accelerate the decay of disinfectants (Zhang and Edwards, 2009; Zhang et al., 2010). Depletion of disinfectant residuals by iron will leave downstream components vulnerable to microbial regrowth. Depletion of chlorine in general (Zahran et al., 2018) and by iron corrosion specifically (Rhoads et al., 2017a) has been hypothesized to account for the Legionnaires' disease outbreak that occurred when corrosive water was distributed in Flint, Michigan. Thus, addressing the problem of legacy iron pipe is a critical engineering control to consider for *Legionella*. In 2012, the American Water Works Association estimated that it would cost $455 million to replace just the cast iron pipe in U.S. distribution systems (AWWA, 2012). In the meantime, awareness of the presence of iron pipes and other components and practicing appropriate corrosion control, e.g., through orthophosphate addition federally mandated by the Lead and Copper Rule, are key to reducing this potential risk factor for *Legionella* growth in premise plumbing.

Finally, other plumbing materials besides the pipes themselves can potentially influence *Legionella*. For example, certain pipe gaskets and elastic sealants (containing polyamide and silicone) can be a source of nutrients for bacterial proliferation (Colbourne et al., 1984).

Managing the Distal Portion of the Plumbing

Managing the distal portion of premise plumbing is the last opportunity to control *Legionella* risk in building water systems. The distal section between the main piping of a building and the point of use has a number of unique features that are favorable to biofilm and *Legionella* growth. Unlike the main and secondary piping, the distal section immediately upflow of the point of use may include numerous components such as faucets, showerheads, thermostatic valves, backflow valves, interconnection piping, and aerators. Because of all these components, the materials found at distal sites vary extensively compared to the main premise plumbing system. In addition, the smaller diameter piping and correspondingly larger surface-to-volume ratios at distal sites provide niches for biofilm growth. These sites are also subject to recurring stagnation, which hinders the maintenance of control measures such as temperature or residual disinfectants. Together, these factors create opportunities for *Legionella* to thrive at distal sites.

There is strong evidence that concentrations of *Legionella* in the distal sites of premise plumbing can be significantly higher than in the more centralized sections of the premise plumbing of a building. Using monitoring data required by German regulations, a large investigation in Cologne focusing mostly on residential buildings revealed that 32.7 percent (223 of 712) of samples were positive for *Legionella* spp. (Kruse et al., 2016), with most positive detections (63.9 percent) found only at distal sites, rather than in

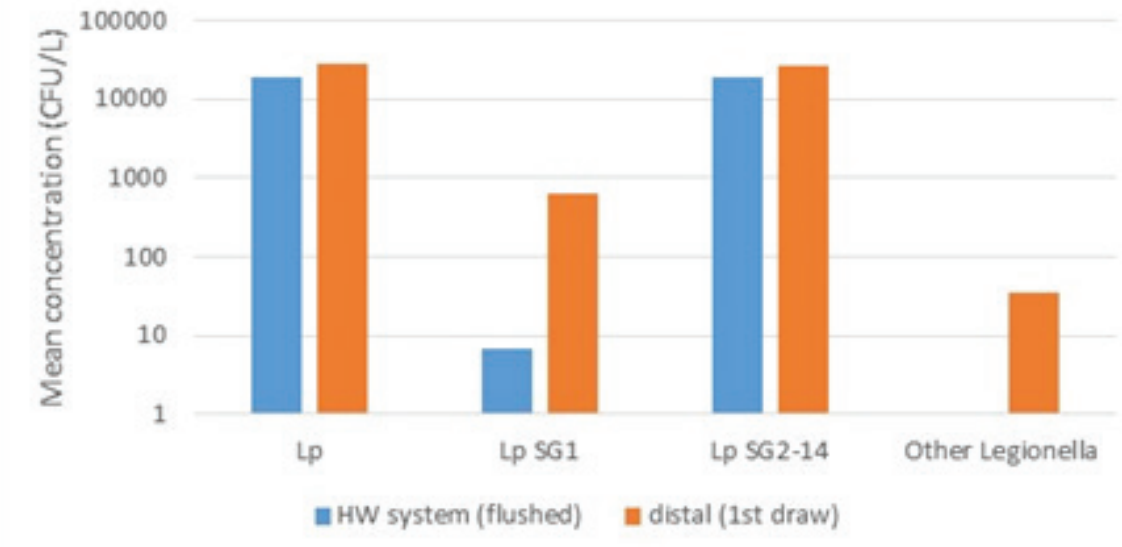

FIGURE 4-3 Concentrations of various *Legionella* species and strains in Italian hospital water systems. First draw samples reflect conditions at the distal ends, while flushed samples represent conditions in the main hot-water system. SOURCE: Cristina et al. (2014).

the central recirculation system. Similarly, a large Italian database of regulatory sampling results for the monitoring of *Legionella* spp. and *L. pneumophila* in hospitals in first-draw and flushed samples was analyzed by Cristina et al. (2014), who found high average concentrations of various *Legionella* strains and species both in the main hot-water plumbing and in first draw samples at taps. As shown in Figure 4-3, significant amplification was noted for *L. pneumophila* serogroup 1 and other *Legionella* in first-draw samples, which specifically measure concentrations in the distal sites.

Biofilm growth and *Legionella* proliferation at distal sites can be prevented through various actions. Small diameter piping in the distal portion of premise plumbing can minimize water volumes and their age. Water circulation can be maximized by a combination of improved design (e.g., limiting the number of outlets) and preventive flushing procedures. The use of biostable materials (see previous section on plumbing materials) and minimization of the surface area available for biofilm growth should also be considered when selecting any distal devices, including faucets and flow-reduction aerators. Finally the use of thermostatic valves, which provide surfaces for biofilm growth at temperatures optimal for *Legionella*, should be carefully weighed against the risk of scalding and only used when justified on a risk basis. In cases where the premise plumbing is compromised, corrective action can be taken by installing point-of-use filtration barriers or flash disinfection devices.

Challenges of Thermostatic Mixing Valves and Electronic Faucets

Electronically activated faucets and thermostatic mixing valves increase *Legionella* risk because they provide surfaces for biofilm growth and water at ideal temperatures (42°C to 49°C) for *Legionella*. Thermostatic mixing valves, mixing manual faucets, and electronic faucets are complex devices composed of various combinations of synthetic, organic, and metal-based materials, often with multiple nooks and crevices where biofilm and *Legionella* can proliferate.

Used mainly in showers and faucets to prevent scalding, thermostatic mixing valves combine hot and cold water to achieve a set temperature that can be adjusted to protect users. There is limited

information available on the impact of thermostatic mixing valves on the prevalence of *Legionella* at the point of use. In The Netherlands, thermostatic mixing valves in hotels and hospitals previously found positive for *Legionella* spp. were investigated in detail (van Hoof et al., 2014). Biofilm swabs and water samples (cold, hot, and mixed) were collected from two types of thermostatic mixing valves, and *Legionella* was quantified both by culture and qPCR. In seven instances, *Legionella* spp. were detected in at least one sample, with swab samples taken from rubber components of the valves showing the highest concentrations, which is in agreement with the high potential of rubbers to support growth of *L. pneumophila* (Niedeveld et al., 1986).

The interplay among materials, water quality, and temperature was investigated at the pilot scale by testing the impact of shower-faucet materials and iron-rust deposits on the growth of *L. anisa* in the absence of any chlorine residual (van der Lugt, 2017). Three types of shower faucets were tested: a faucet with a stainless-steel 304 housing and a ceramic mixer, a brass housing with a ceramic mixer, and a brass thermostatic mixing valve faucet. Increasing levels of positivity were observed for the stainless-steel faucets (14.3 percent), the brass (32.1 percent), and the faucet with the thermostatic mixing valve (85.7 percent), and adding iron rust deposits collected from a building water tank increased the maximum *L. anisa* concentrations observed. These results suggest that thermostatic valves are the faucet type most vulnerable to *Legionella* contamination and that iron corrosion byproducts can enhance the potential for *Legionella* spp. proliferation in faucets.

Several approaches can minimize the impact of thermostatic mixing valves including changing their configuration, placing them as close as possible to the point of use, avoiding all dead volumes such as bypasses, providing ready access for maintenance and cleaning, and selecting valves made of materials that do not support biofilm growth and that can withstand elevated temperatures and oxidants for disinfection. Within thermal mixing valves, integrated check valves prevent backflow into cold- or hot-water feed piping. Unfortunately, some of these check valves are susceptible to breakage and fouling. Their failure results in the mixing of cold and hot water in the piping, which leads to poor service and temperature conditions favorable to the growth of *Legionella* (Boppe et al., 2016). Several guidance documents specify the maintenance and even the installation of backflow valves (Castex and Houssein, 2005). Many guidelines and regulations require the use of thermostatic mixing valves only if needed based on a scalding risk assessment (e.g., Government of South Australia, 2013; HSE, 2013a).[5]

Electronically activated faucets have been linked to greater risk of contamination by premise plumbing pathogens, including *Legionella*, and have been shown to be the cause of several nosocomial outbreaks (Charron et al., 2014; Leprat et al., 2003; Moore and Walker, 2014; Yapicioglu et al., 2011). Sydnor et al. (2012) showed that nearly all electronic-eye faucets were colonized by *Legionella* spp. compared to only 45 percent of manual faucets. More importantly, the electronic-eye faucets were more resistant to disinfection by chlorine dioxide (Sydnor et al. 2012). Importantly, electronically activated faucets typical contain thermostatic mixing valves and flow-reducing devices such as complex aerators. Bacterial colonization of such faucets results from the tepid water temperature, type of materials used, and the lower flows typical of these devices (Charron et al., 2015).

Terminal Tap Water Filters

Different types of terminal filters, often referred to as point-of-use (POU) filters, are available commercially and can be installed either at faucets or retrofitted to showerheads to prevent exposure in high-risk patient care areas. Such filters, typically of 0.2-μm porosity, provide a physical barrier to *Legionella*, are disposable, and are sometimes impregnated with biocides.

[5] See https://www.cdc.gov/legionella/wmp/ monitor-water-guidance.html.

Many studies mention the high cost of these filters, driven by the large number of devices that may need to be installed and their relatively short life (eight to 30 days) before clogging or breakthrough (Marchesi et al., 2011; Sheffer et al., 2005; Zhou et al., 2014). In the same hospital complex in France, three POU shower filter devices showed wide ranges of use before clogging, ranging from three days to more than six months (Lecointe et al., 2010). Some reports show either low-level breakthrough or a return of contamination after one week of use (Vonberg et al., 2005) or 12 weeks (Baron et al., 2014). The time before clogging is dependent on the type of POU device and on the nature of the feed water.

In a cancer center in Pennsylvania, a new extended-life faucet filter ensured total removal of *Legionella* spp. for 12 weeks, exceeding the recommended period of use of 62 days, while mean concentrations at control faucets ranged from non-detect to more than 600 CFU/mL (Baron et al., 2014). A multi-layer design including two pre-filters of 30- and 1-mm porosity resulted in minimal flow restrictions and extended the life of the devices, halving the number of change-outs and associated costs. Recently, an electrically heated carbon nanotube and polymer membrane POU filter were proposed to inactivate any captured bacteria by increasing temperature on the membrane to 71°C to 83°C (Oh et al., 2019). Although this new membrane-interface POU removed 99.99 percent of *L. pneumophila*, further validation is warranted. Extreme care must be taken to ensure that water pressures at POU filters do not exceed manufacturer's recommendations. Pressures in excess of ratings can cause filter media to break away and release contaminated water at the distal device.

Showers and taps have been designed and fitted with UV lamps located immediately before the outlet for microbial control and have been installed in a number of hospitals in the UK (Moore and Walker, 2014), but their efficacy remains to be seen. Using on-site UV treatment on the incoming water main was credited for avoiding any positive detects of *Legionella* in a new hospital and for the lack on any documented Legionnaires' disease in the subsequent 13 years (Hall et al., 2003), although critical information about system hydraulics and other treatment was not provided.

Aerosol Formation Prevention

Aerosol formation is a critical risk factor in the transmission of legionellosis (Hamilton et al., 2018a). Therefore, preventing or reducing their formation can be an effective strategy for managing *Legionella* risk. Laminar flow of water is preferred, as devices that intentionally break the water stream (e.g., shower nozzles, faucet aerators, spray nozzles) can create respirable droplets less than 5 μm (see Figure 4-4; ASHRAE, 2000). Therefore, aerators should be removed from faucets to create a laminar flow (enHealth, 2015). Falkinham (2013) recommends the following to reduce aerosol exposures in the bathroom: (1) replace a showerhead with one that produces water streams (holes larger than 1-mm diameter) rather than a fine mist, (2) replace a showerhead with one that contains a microbiological filter (i.e., pore size less than 0.45-μm diameter) to reduce the proportion of aerosol droplets containing bacteria that can enter the lung, (3) open a window in the bathroom (if possible), (4) replace an inefficient fan with one that exhausts bathroom air rapidly, and (5) minimize the time that bathroom aerosols are created, for example, by shortening showers.

Cooling towers and evaporative condensers incorporate drift eliminators to remove water droplets generated within the units (e.g., CoolClean, 2019; VisTech, 2019). The main purpose of these devices is to collect water droplets on a surface, which then directs the water back to the cooling tower. Newer design standards can reduce the drift to a maximum of 0.0005 percent of the cooling tower flow (Stodlka and Vitkovi, 2016). The humidity of the air, however, can cause larger droplets to be reduced by evaporation to 5 μm or less. At wastewater treatment plants, changes in aeration technology (e.g., use of fine bubble diffusers) or covering the aeration basins can reduce aerosol formation and transport (Prussin et al., 2017).

Standard Aerator
Draws up to 50%
Produces Aerosols
Bacterial growth on screen

Spray Aerator
Produces a small shower
Produces Aerosols
Bacterial growth on screen

Non-Aerated Laminar Flow
Does not mix air and water
Reduced aerosols
No surfaces for growth

FIGURE 4-4 Examples of faucet aerators and impact on aerosol Formation.
SOURCE: https://www.plumbingsupply.com/water-saving-low-flow-aerators.html.

HOW THESE CONTROLS ARE APPLIED TO SPECIFIC SYSTEMS

The strategies discussed above can be applied in various ways to all of the major building water system types for the purpose of *Legionella* control. Table 4-1 provides an overview of the type of controls relevant to particular systems, categorizing them as (1) large engineered systems (potable water supply, wastewater treatment facilities, water reuse systems); (2) building water systems (large buildings, households, green buildings); and (3) other devices (cooling towers, humidifiers, hot tubs). The following sections provide an overview of how the various control strategies are or are not applied to each system in theory and in practice. Legal frameworks and guidance documents addressing these various systems will be covered in Chapter 5. In general, whether a water system presents a potential risk as a *Legionella* source and requires control depends on the following criteria (HSE, 2013b):

- Presence of *Legionella* in the system water;
- Water temperature between 20°C to 45°C;
- The system has the means to create and/or spread aerosols;
- The system stores and/or re-circulates water;
- The system is likely to contain a source of nutrients for *Legionella*, such as contaminants from the surroundings or from the process, including the presence of sludge, rust, scale, organic matter, or biofilm.

Public Water Supplies

Public water supply is an important consideration in *Legionella* management, as the characteristics of the water chemistry will vary seasonally and regionally, depending on drinking water source. The local water supply will be characterized by varying degrees of hardness, corrosivity, and nutrient content, which in turn impacts disinfectants and plumbing materials. Correspondingly, distinct microbiomes

have been noted in controlled premise plumbing pipe rigs as a function of the local water chemistry (Ji et al., 2015).

In theory, public water supplies that already comply with local, state, and federal safe water regulations and implement standard practices, including maintaining a disinfectant residual, hydraulic control via routine flushing, and cleaning of storage tanks, have a strong foundation for controlling *Legionella* risk. The underlying statutes of these regulations and practices were developed to provide protection from a wide range of chemical and microbiological hazards. Because of cold water temperatures and the presence of a disinfectant residual, public water distribution systems are generally thought to harbor low levels of *Legionella*, although there are few data to support this assumption. Continued emphasis on the following elements is essential for reducing exposure to *Legionella* from public water supplies.

Control Options

Disinfection. Most water utilities in the United States strive to maintain a minimum of 0.2 mg/L disinfectant residual in all parts of the pipeline system (AWWA, 2018). The voluntary Partnership for Safe Water program, for example, requires that all member systems use secondary disinfection and that "optimized" systems meet these residual disinfectant goals throughout the distribution system:

- ≥ 0.20 mg/L and ≤ 4.0 mg/L for free chlorine,
- ≥ 0.50 mg/L and ≤ 4.0 mg/L for total chlorine (chloramines),
- ≥ 0.20 mg/L and ≤ 0.80 mg/L for chlorine dioxide.

The goals are to be achieved for 95 percent of the routine readings each month, and individual routine sample sites should not have consecutive residual readings less than the residual disinfectant goal. Additionally, well-run systems specifically target areas known to experience low disinfectant residuals due to the pipe materials (e.g., unlined cast iron mains), long retention times, or water quality characteristics (e.g., organic matter, inorganic chemicals, pH, temperature). In these cases, the stability of the disinfectant residual can be increased by replacing old mains, improving the circulation within the distribution system, or improving treatment processes.

To improve control of *Legionella*, EPA has proposed to review the Surface Water Treatment Rule residual disinfectant requirement for "at least 0.2 mg/L at the point of entry and detectable in at least 95 percent of samples collected within the distribution system."[6] Several papers suggest that disinfectant residuals are lost once water starts to stagnate in premise plumbing (Bédard et al., 2018; Charron et al., 2015; Prévost et al., 1997). Additional research is needed to understand the persistence of distribution system disinfectant residuals within building plumbing. This is important not only for large buildings where it is often assumed that residuals are insufficient to affect *Legionella*, but also for single-family homes and small buildings, where there is little solid information on the persistence of residuals.

Hydraulic Management. Public water systems should have a routine program for systematically flushing and cleaning the distribution system, as over time bacterial growth can be promoted by precipitation of treatment chemicals, settling of fine silt, and corrosion products that form sediments within the pipelines. Implementation of a "uni-directional" flushing program is recommended, during which hydrants are opened near the treatment plant, and water is flushed systematically away from the plant toward the ends of the system; this approach avoids recirculating water from unflushed pipes into the cleaned sections of the system. Application of a hydraulic model is useful to ensure that adequate water pressure is maintained while achieving the targeted velocity (greater than 5 ft/s) (Friedman et al., 2002).

[6] See https://www.epa.gov/dwsixyearreview/six-year-review-3-drinking-water-standards.

Hydraulic management of the distribution system is also important to avoid areas of water stagnation that can result in the loss of a disinfectant residual and the potential for regrowth. Areas of greatest concern are dead-end or dead-leg sections of pipes (e.g., at the ends of a pipeline where there is no circulation), inadequate mixing in storage reservoirs and tanks, and areas of the distribution system with poor circulation. Distribution system hydraulic models can identify these stagnant areas to evaluate options to mitigate. Water circulation is often improved by creating loops in the pipe system, avoiding closed valves, and installation of automatic flushing valves (NAS, 2006).

Like distribution system pipelines, sediments and corrosion products can accumulate in storage tanks, which require periodic inspection and cleaning. *Legionella* spp. have been detected by qPCR in 66.7 percent of municipal drinking water storage tank sediments from 18 sites (Lu et al., 2015). The AWWA Manual M42 (AWWA, 2013) recommends that tanks be drained and inspected at least once every three years or as required by state regulatory agencies. Periodic inspections by operators are recommended more frequently (monthly or weekly) and can be aided by drone technology to alleviate the need for a person to climb the tank. Water quality in the tank can be improved by installation of devices to ensure water circulation and to prevent stratification, stagnation, and loss of the disinfectant residual (EPA, 2002a).

Nutrient Limitation. Nutrient limitation in public water supplies includes reducing nutrients during water treatment, corrosion control, and preventing nitrification in the distribution system. Biological filtration treatment processes (e.g., rapid sand filtration for groundwater treatment and biological active carbon filtration and slow sand filtration for surface water treatment) are pivotal for nutrient removal during drinking water treatment. Controlling corrosion of cast iron pipes in the distribution system prevents iron from leaching into the environment, which can limit growth of *L. pneumophila* because iron is an essential nutrient. Finally, when the chlorine-to-ammonia ratio (4.5:1) is not properly managed in chloraminated drinking water, nitrification can occur, enhancing biofilm biomass and increasing the number of protozoan hosts for *L. pneumophila*.

Plumbing Materials. Most public water supply distribution systems consist of hundreds of miles of cast-iron mains, which will never be replaced in a time frame that would allow for better *Legionella* control. Even where plastic pipes have been installed, metal hydrants, valves, and other appurtenances remain. Pipes, valves, gaskets, coatings, and other materials that contact public drinking water supplies must be approved for use according to NSF/ANSI 61: Drinking Water System Components–Health Effects.[7] Unfortunately, the NSF/ANSI 61 standard does not address the microbial growth potential of materials in contact with water, unlike similar standards in Europe (van der Kooij et al., 2003; Prest et al., 2016 a,b). Further, it is not simple for water utilities to change the materials already present in their distribution systems. However, NSF/ANSI 61 could implement standards to reduce microbial growth on water-contact materials so that utilities have better information in the future.

For many utilities, corrosion control is implemented in compliance with the Lead and Copper Rule (EPA, 1991). However, these procedures may not be sufficient to address corrosion of other metallic materials.

Temperature Control. It is impractical for most public water systems to effect major changes in water temperature in their distribution systems, but there are practices that can be used by some utilities to impact water temperature. For example, intakes can be positioned below the thermocline in some raw water supplies, so that the cooler source water can be withdrawn. In some systems, warm surface waters can be blended with cooler groundwater supplies. Management of water mixing and turnover in elevated storage tanks can prevent water stratification during warm weather and help to control water temperature and disinfectant residual loss (Peter and Routledge, 2018).

[7] See http://www.nsf.org/services/by-industry/water-wastewater/municipal-water-treatment/nsf-ansi-standard-61.

Reclaimed Water Systems

Reclaimed water is municipal wastewater treated to high standards for beneficial use such as drinking water or irrigation water (EPA, 2012). This is a growing practice that presents many advantages, especially reducing water demand in arid and drought-prone regions as well as avoiding negative consequences of unintended, *de facto* reuse (NRC, 2012). A challenge with reclaimed water is that the level of treatment is dictated by the particular application. For direct or indirect potable reuse, the level of treatment often surpasses that for conventional drinking water treatment. In these instances, the control measures for *Legionella* would be similar to those outlined above for public water systems.

Reclaimed water treated for unrestricted reuse refers to non-potable water used where public access is not restricted. Water classified for unrestricted urban reuse is commonly applied for spray irrigation on parks, playgrounds, schoolyards, and residences, and for other applications such as toilet flushing, air conditioning, fire protection, construction, ornamental fountains, and other water features. *Legionella* has been routinely detected in many unrestricted reuse systems (Ajibode et al., 2013; Birks et al., 2004; Buse et al., 2015; Garner et al., 2018; Jjemba et al., 2010; Johnson et al., 2018). These systems typically do not maintain a disinfectant residual nor are they routinely flushed or cleaned. Jjemba et al. (2010) described the characteristics that contribute to the growth of microbes in reclaimed water distribution systems, including warm temperatures, elevated levels of biodegradable organic carbon and other nutrients, loss of disinfectant residuals, and variable use patterns that lead to stagnation and depressurization, among others. A recent survey of four reclaimed water distribution systems indicated elevated *Legionella* gene markers at the point of use, compared to paired potable water systems monitored in the same study (Garner et al., 2018). Brunkard et al. (2011) reported one outbreak of Legionnaires' disease associated with use of reclaimed water at a mass-transit vehicle washing station. Hamilton et al. (2018a) reported that risks of *Legionella* exposure from reclaimed water used for irrigation or cooling towers could exceed 10^{-4} annual risk of infection for various scenarios. A review by Garner et al. (2016) highlighted that reclaimed waters are very different from traditional potable waters in terms of water quality, conveyance practices, exposure routes, and health risk. Because distinct water chemistries could place reclaimed water plumbing in uncharted territory for *Legionella* control, the authors call for water quality management guidelines and regulations more specifically tailored to recycled water. Jjemba et al. (2015) reported on best management practices (BMPs) for maintaining water quality in reclaimed water systems (see Box 4-1). Many of these BMPs are similar to those mentioned for public water systems (e.g., optimizing water age, managing storage, corrosion control, biofilm control, etc.), but managing risk of inhalation, rather than ingestion, needs to be emphasized.

Treating recycled water for purposes of direct potable reuse is gaining momentum. For example, a 2 million gallon per day direct potable reuse plant in Big Spring, Texas, treats wastewater to drinking water standards via microfiltration, reverse osmosis, and UV disinfection before blending with raw drinking water sources and routing to a conventional drinking water treatment plant (Trussell et al., 2015). Given that such an approach meets current drinking water standards, there should not be any special concerns related to *Legionella* beyond that of a typical municipal water supply. Nonetheless, out of an abundance of caution, efforts are underway to understand how blending of direct potable reuse water with conventional water supplies and treatments may adversely affect distribution systems via corrosion and other processes (Water Research Foundation, 2018). A pilot-scale survey following incubation of a range of direct potable reuse blends from different utilities in PVC pipe over eight weeks indicated only rare detection of *Legionella* spp. gene markers by qPCR (Garner et al., 2019). While this result is encouraging, longer-term studies and monitoring are recommended as municipalities begin blending direct potable reuse water.

BOX 4-1
Best Management Practices for Reclaimed Water Systems

1. Optimizing reclaimed water storage
2. Minimizing the impact of reclaimed water corrosivity
3. Improving customer perception
4. Managing reclaimed water total dissolved solids (TDS)
5. Controlling algae in reclaimed water reservoirs and distribution systems
6. Managing snails and other macroorganisms in reclaimed water
7. Minimizing regrowth, odor and biofilms in reclaimed water systems
8. Monitoring of cross-connection control
9. Managing reclaimed water age to enhance quality and operational bottlenecks
10. Ensuring pressure sustaining reclaimed water systems
11. Staying within reclaimed water turbidity targets
12. Operational management of reclaimed water supply and demand challenges
13. Monitoring the distribution system
14. Considering emerging contaminants in reclaimed water

SOURCE: Adapted from Jjemba et al. (2015)

Wastewater Treatment Plants

Wastewater treatment plants, especially those with biological treatment processes, can be a source for *L. pneumophila* (Caicedo et al., 2019). What measures can be taken to control legionellae depends on the treatment process in the wastewater treatment plant. For example, certain aerosol-producing installations at treatment plants (e.g., air scrubbers) can be controlled by disinfection using hot steam or hypochlorite treatment (Olsen et al., 2010). Norway is one of the few countries where control measures for aerosol-producing devices in wastewater treatment are regulated.

In most outbreaks involving wastewater treatment plants, the biological treatment process is identified as the main cause for *L. pneumophila* growth (Caicedo et al., 2019). Control measures that are normally taken against legionellae (e.g., thermal control, chemical disinfection) are difficult to implement in biological treatment processes because these control measures will also eradicate the microorganisms that treat the wastewater. In addition, laboratory experiments have shown that disinfection of wastewater effluent with chlorine dioxide, hydrogen peroxide, silver ions, ozone, and alkalinization did not result in reduction of cultivable legionellae (Noguiera et al., 2016). As a result, alternative control measures have been implemented at plants that have been identified as the source for an outbreak of Legionnaires' disease or Pontiac fever. At locations where only workers became infected with *L. pneumophila*, workers were required to wear respirators that prevent inhaling of aerosols and/or prevent use of *L. pneumophila*-contaminated waters for cleaning purposes (Castor et al., 2005; Gregersen et al., 1999; Kusnetsov et al., 2010).

Different control measures have been taken at wastewater treatment plants where the biological treatment process (e.g., an aeration pond) was identified as the source for *Legionella* infection among residents who live in the vicinity of the plant. One example is a plant in Norway that treated wood refinement waste. As the ultimate infection control measure, this plant was shut down, but its organic content was then released into the river (Borgen et al., 2008). During a large Legionnaires' disease outbreak in Warstein, Germany, several control measures were implemented at the biological wastewater treatment

plant that was the primary source of the outbreak (Noguiera et al., 2016). UV was installed to treat the effluent before it was discharged into the river, which resulted in a 1.6- to 3.4-log reduction of legionellae in the effluent (from about 10^6 CFU/L to about 10^4 CFU/L). Second, the aerobic pre-treatment process was stopped, which resulted in a significant decrease of *L. pneumophila* in the wastewater (to 10^2 CFU/L) and in the effluent (below the detection limit). Finally, measures were taken to reduce aerosol emission from the wastewater treatment plant, although these measures were not specified. In the Netherlands, control measures at two biological treatment plants that were involved in small outbreaks of *L. pneumophila* focused on preventing aerosolization from the aeration ponds to the open air. This was done by successively erecting tents to cover the aerated ponds in combination with ventilation to prevent overpressure in the covering tents (Loenenbach et al., 2018).

Large Buildings

Large buildings include most hospitals and many long-term care facilities, as well as apartment complexes, hotels, offices, high rises, schools, prisons, and industrial complexes. *Legionella* is inherently more difficult to manage in larger building water systems because the plumbing networks are correspondingly larger and subject to more variability, making it more challenging to ensure that controls are adequately supplied throughout the building. The extended stagnation periods experienced by water in large building premise plumbing place these systems at further risk. Thus, *Legionella* management in large buildings tends to focus on thermal control (Bédard et al., 2015; Boppe et al., 2016) or on-site disinfection. Any controls that have been emplaced on the municipal water supply up to the property line, e.g., a minimum chlorine residual of 0.2 mg/L, are unlikely to provide reliable protection throughout a large building plumbing network.

Another challenge is that large buildings often require substantial water storage for water security purposes. However, during storage the water quality can degrade substantially, posing problems in times of need. Large buildings also often employ potable or recycled water for other purposes, including humidifiers, landscape irrigation, decorative fountains, hot tubs, swimming pools, and cooling towers to manage extensive HVAC needs. In this section the emphasis is on piped potable water used for drinking and bathing, though general principles apply to other piped water systems. Cooling towers, humidifiers, and hot tubs are discussed in separate sections.

Design and commissioning of a large building is a key opportunity to ensure that *Legionella* control is prioritized, including appropriate design and implementation of hot- and cold-water systems and HVAC features. Further, large building water systems should be configured to facilitate collection of water for *Legionella* monitoring as well as implementation of maintenance and remediation (e.g., sampling and injection ports on hot-water lines). Hospitals or other buildings where sensitive populations are housed should be designed to facilitate remediation in the case of contamination by *Legionella* or other pathogens. Unfortunately, in reality the majority of existing large buildings were not designed in this manner and present numerous complex challenges for *Legionella* control.

Much of what has been learned to date about management of *Legionella* in large buildings comes from hospitals. Table 4-5 summarizes long-term hospital experience with various combinations of disinfection and thermal regimes, including long-term studies (up to ten years) with extensive monitoring to support findings. From these data, the two controls that emerge as being most broadly effective are (1) temperature set points of greater than 60°C at the water heater and greater than 55°C in the recirculation loop and (2) chloramine as an on-site disinfectant. Combining elevated temperature with addition of disinfectants yielded the best results in some cases.

TABLE 4-5 Long-term Hospital Experience Using Multiple Strategies for the Control of *Legionella*

Type of facility	Permanent regime	Shock disinfection	Targets	Findings	References
Hospital with 1,077 beds 9 years	ClO_2 for greater than 9 years Chloramines – 26 weeks	None	*Legionella* spp. and Mycobacterium avium complex (MAC)	ClO_2 for greater than 9 years reduced *Legionella* positivity to 51% but with high concentrations (greater than 10^5 CFU/L) remaining at some sites. Chloramines at 2 mg/L for 26 weeks reduced all sites from greater than 10^3 CFU/L to non-detect but increased positivity for MAC.	Casini et al. (2014)
Hospital with four circuits (A-D) 5-18 years with *Legionella* contamination	A: >55°C -60°C B: >65°C one day per quarter C : A&B + chlorine D: chlorine	A. 30-min 70°C heat shock twice per year B. three 30-min 70°C heat shocks, then none C. none D. chlorine	*Legionella* spp. and *L. pneumophila*	Frequent heat-shock treatment (65°C) develops temperature resistance in some *Legionella* strains. The combination of maintaining 65°C one day per quarter combined with chlorine was more efficient than repeated heat shocks at 70°C.	Allegra et al. (2011)
Hospital with three wings	ClO_2 two types reactors– 1 year Chloramines – 1 year	ClO_2: 0.6 to 0.9 mg/L Chloramines: 2 mg/L and 3 mg/L	*L. pneumophila* and *Pseudomonas aeruginosa*	ClO_2: positivity went from 100% to 57-61% ClO_2 less effective for *L. pneumophila* sg1 With chloramines, quick (<1 month) and large reduction in # positive sites and concentrations of *L. pneumophila*. 2 mg/L chloramines: < 100 CFU/L for *L. pneumophila*; 3 mg/L chloramines: below detection limit for *L. pneumophila*. Both disinfectants reduced *P. aeruginosa*.	Marchesi et al. (2012)
Two hospitals A: 255 beds B: 450 beds	A: sub-optimal thermal regimes and Cu-Ag ionization B: suboptimal thermal regime	None	*Legionella* spp. *L. pneumophila*	Need for online temperature monitoring for system characterization Large differences between sectors Poor thermal regime results in greater *Legionella* and *L. pneumophila* prevalence Framework for risk analysis using temperature profiling	Bédard et al. (2015)

TABLE 4-5 Continued

16 hospitals 5 years	Cu-Ag ionization	50% used superheat/ flush and 31% used hyperchlorination	*Legionella* spp. *L. pneumophila* sg1 *L. pneumophila* sg2-14	50% of sites reported zero positive samples in the years after implementation 5% hospitals reported cases of nosocomial legionellosis (from 100% before)	Stout and Yu (2003)
Hospital with 450 beds 11 years	Increasing temperature from 45°C to 65°C with distal temperature of 56°C - 61°C for 5 minutes after outbreak	Weekly flushing of all taps and showers at 65°C for 5 minutes over 18 months	*Legionella* spp. *L. pneumophila* sg1 *L. pneumophila* sg2-14	Striking decrease from ~100% to less than 20% samples positive with lower concentrations of *L. pneumophila* sg1 (<500 CFU/100mL) Increasing temperature arrested Legionnaires' disease outbreak, but four cases occurred during 10 years even with only 5% positivity.	Darelid et al. (2002)
Two hospitals A: 364 beds B: 672 beds 2 years	ClO_2 dosages of 0.5-0.7 mg/L in cold water	Not specified	*Legionella* spp. Heterotrophic plate counts	Reduced positivity from 60% to less than 10% Increased again after 2 years Decreased heterotrophic plate counts	Zhang et al. (2009)
Hospital with 1,266 beds	Target dosages of Cu/Ag 0.2/0.02 mg/L Cu/Ag concentrations at distal sites mean 0.16/0.014 mg/L	2 superheat/flush treatments	*Legionella* spp.	Positivity in intensive care units of 14% and 66% after two superheat/flush treatments Low Cu-Ag dosages were not effective Increased Cu-Ag dosages lowered positivity to 0-5% No nosocomial cases after implementation	Chen et al. (2008)
Hospital with 765 beds	ClO_2 dose of 0.3 mg/L distal concentration for 9 years Monochloramine at 3 mg/L for one year	Superheat (>60°C) for 2 days, 8 times in 4 years Hyperchlorination (20-50 mg/L) for 1-2 hours, 12 times in 8 years	*Legionella* spp. *L. pneumophila*	Superheat treatment led to an insignificant temporary reduction Hyperchlorination effective for 2 months ClO_2 reduced positivity from 97% to 54% *Legionella* spp. but less for *L. pneumophila*	Marchesi et al. (2011)
57 buildings	Conversion from free chlorine (0.6 mg/L) to monochloramine (1.9 mg/L) in the distribution system Only 13% of water heaters >60°C	None	*L. anisa*, *L. bozemanii*, *Legionella* spp., *L. pneumophila* sg1 and 2-14	Increased *Legionella* colonization was observed in buildings with temperature less than 50°C Chloramines strikingly (60% to 4%) and rapidly (<3 months) reduced the percentage of sites positive for *L. anisa*, *L. bozemanii*, *Legionella* spp., *L. pneumophila* sg1 and 2-14	Flannery et al. (2006)

Control Options

Temperature Control. Maintaining a high water temperature (ideally greater than 60°C) in hot-water lines is the primary line of defense against *Legionella* in large buildings. This can be accomplished in part by installing multiple water tank heaters. Recirculating lines are also commonly employed to ensure delivery of hot water throughout the building. Recirculating lines are susceptible to heat loss and can readily fall into the ideal temperature range for *Legionella* growth if not maintained at a sufficiently high temperature (Brazeau and Edwards, 2013a,b,c). This can be avoided by insulating recirculating pipes (as required in Canadian building codes for large buildings) and making sure the recirculating velocities are sufficiently high. Recently, the California Exchange Commission has mandated insulation of hot-water lines (CEC, 2019). Despite the challenges mentioned above, maintaining water above 55°C across the whole system in large buildings with multiple recirculation loops can be done at relatively low cost (Bédard et al., 2015, 2016). Successful and low-cost hospital interventions have shown that poor temperature maintenance can be corrected by removing dead-end pipes, inadequate heat exchangers, and faulty thermostatic valves that can cause flow inversions and mixing with cold water (Bédard et al., 2016; Boppe et al., 2016; Lecointe et al., 2018).

Likewise, temperatures of cold-water lines can also increase into the *Legionella* growth range, particularly in warm climates and as water makes its way through extensive distal plumbing within the warmer building envelope. In such cases, cold-water line flushing and pipe insulation to minimize heat transfer to the cold-water piping can help. In a study of a major hospital in Germany, the cold-water lines were as contaminated with *Legionella* as the hot-water lines, and 35 percent were positive, even at sites where the measured temperature was less than 20°C (Arvand et al., 2011).

Disinfection. As mentioned previously, disinfectant residuals from the distribution system may not persist in the premise plumbing of large buildings. Hence, many hospitals and long-term care facilities in particular have found on-site disinfection to be highly beneficial. Disinfectant can be added at a constant level to manage *Legionella* risk, or it can be increased in response to elevated *Legionella* numbers or an outbreak. Low doses of disinfectant are often an effective preventative measure, but much higher doses are required for remediation purposes (see Table 4-5). Disinfection systems are typically added to hot-water lines to avoid any concerns with human consumption, as hot-water lines are not intended to produce water for ingestion, but they can be added to cold-water lines as well. Popular disinfectants for this purpose include chloramine, chlorine, copper-silver ionization, and chlorine dioxide. However, it is important to be aware of the local water chemistry, pipe materials, and other constraints of relying on such disinfectants (Rhoads et al., 2014). For example, iron plumbing components can reduce the chlorine residual by stimulating its decay. During a major Legionnaires' disease outbreak at an Illinois veteran's home in Quincy, reaction of on-site chlorine addition with old iron pipes delivering water throughout the campus significantly depleted chlorine residual (Rhoads et al., 2018). Copper-silver can also lose its efficacy and fail to be delivered appropriately, for example, by plating onto pipe surfaces instead of maintaining dissolved form, if installed incorrectly, or if the water chemistry is incompatible (Triantafyllidou et al., 2016; Walrayen et al., 2016). Chloramine is probably the most popular on-site disinfectant, but its decay can be accelerated by nitrifying microorganisms, which happen to thrive in a similar warm temperature range as *Legionella*. UV can also be applied, but it may be most effective at the point of use since it does not leave a disinfectant residual.

Hydraulic Management. Fundamental to reducing *Legionella* risk is managing the hydraulics of the plumbing system to ensure delivery of both hot water and disinfectant. As discussed previously,

recirculating lines are commonly employed to achieve this purpose. Aside from temperature and disinfectant delivery, maintaining a low water age itself is a key aspect of hydraulic design. Installing flushing devices can help alleviate other water age issues, such as taste, odor, microbial growth, and nitrification (Nguyen et al., 2012). Dead legs and other flow anomalies must be avoided at all cost. For extremely large buildings or other situations where it is difficult to control water age, automatic flushing devices and programs may be beneficial as a routine maintenance or remedial measure. There is no consensus on the optimal frequency and duration of flushing for efficient *Legionella* control, but evidence clearly demonstrates that the use of frequent (one minute every two hours) automated flushing of hot-water taps with low use or poor recirculation (dead-ends) can eliminate *Legionella* positivity (Darelid et al., 2002; Totaro et al., 2018).

Distal Devices and Aerosol Control. Even with maintaining water temperature and disinfectant levels in hot-water lines, it is critical to consider the mode of delivery at the fixture. Benefits and susceptibilities of various faucets and means of delivery were discussed in a previous section. Low-flow fixtures have been promoted to both conserve water and in some cases energy. However, as a consequence of their lower flow, these fixtures, primarily faucets but also showers, increase water age and restrict disinfectant levels, including the disinfection provided by elevated water temperatures. As such, low-flow fixtures present a greater risk for *Legionella* development in the distribution systems that feed them. Low-flow fixtures should be restricted from use in hospitals and long-term care facilities due to their high-risk occupant populations.

As previously discussed, faucets with a "hands-free" designs, including automatic sensors and foot pumps, do not reduce microbial risk (Sydnor et al., 2012). Compared to traditional fixtures, these designs tend to have higher surface area for biofilm formation and are more conducive to *Legionella* growth. The same is true of the thermostatic mixing valves that produce warm water to enhance the comfort of the hand-washer. Faucet and fixture selection is a key decision, as these are typically installed as a building-wide standard.

In extreme cases where *Legionella* growth is uncontrolled and patient populations are extremely sensitive, size exclusion point-of-use ultrafilters can be installed. These will effectively remove *Legionella* from the water at the tap. However, such filters are quite costly and not a sustainable long-term solution. Selecting a device that can function with the incoming water quality, as well as proper installation and maintenance, are key to ensuring cost-effective use and efficacy (Baron et al., 2014).

Households

Households are a poorly understood source of *Legionella* that may contribute to the large percentage of legionellosis cases known to be sporadic (see Chapter 3; Adams et al., 2015; McClung et al., 2017; Shah et al., 2018). While water use in the home is generally more consistent than in public buildings, old piping (e.g., galvanized steel), dead legs, and low-use locations can all provide the opportunity for *Legionella* growth. Additionally, most home hot-water heaters are set at temperatures to limit the risk of scalding but are within the range for *Legionella* growth (see Table 4-3). Travel, hospitalization, home construction and remodeling, and other events that restrict water use can lead to potential opportunities for *Legionella* to proliferate in household water systems. Finally, there is the potential for *Legionella* growth in devices found in homes that are in contact with water, including humidifiers, nebulizers, and hot tubs. Given all of these potential sources of contamination, a key risk prevention strategy at the household level is communicating the risks of *Legionella* to immunocompromised individuals and to those who purchase devices for in-home use that could create aerosols containing *Legionella*.

Households may be served by a public water supply or by private wells. An estimated 13 million households rely on private wells for drinking water in the United States (U.S. Census American Housing Survey, 2017 data[8]), but EPA regulations do not apply to these private systems. Little information is available about occurrence of *Legionella* in private well water (Stojek and Dutkiewicz, 2011). Well water is not routinely disinfected, which could potentially leave well owners more susceptible to *Legionella*. For example, in a large field study of 255 domestic water heaters, those fed by a groundwater source distributed without any treatment were more often positive (46.3 percent) than water heaters supplied by surface water sources with residual chlorine (26.2 to 27.5 percent) (Dewailly and Joly, 1991). Most groundwater supplies in the United States would be considered low risk because of their cold temperature, but there are areas of the country where groundwater may be warm enough to support *Legionella* growth (Riffard et al., 2001). However, for most private systems, management of *Legionella* risk is mainly associated with managing the hot-water system and the devices in the home that come into contact with water and produce aerosols, as described below for typical households. Intrusion of soil and other contamination that could contain *Legionella*, particularly as a result of major weather events, also require attention. Well owners are given general guidance on how to remediate such intrusion events, typically by addition of bleach (i.e., chlorination) as a shock treatment. Specific guidance on *Legionella* control is needed for well owners, especially for immunocompromised individuals.

Control Options

Most of the strategies summarized in Table 4-1 could play a role in managing *Legionella* in households, though homeowners seldom implement them formally.

Hydraulic Management. To prevent biofilm growth and exposure to *Legionella*, homeowners are recommended to perform several maintenance activities, including the regular flushing of sediments from hot-water tanks and cleaning of faucet aerators, showerheads, hot tubs, nebulizers, evaporative cooling fans, and humidifiers (Leoni et al., 2018). Guidance is also provided in a recent Water Research Foundation report (#4664—Customer Messaging on Plumbing System Issues) that developed materials for water utility websites.[9]

Because smaller-diameter pipes are found in buildings and homes, premise plumbing is particularly prone to growth of biofilm bacteria and resulting water quality problems. Although there are no ways to reduce the nutrient content of water entering premise plumbing, other strategies can be employed to control biofilm growth, including flushing pipes to reduce water age and deliver disinfectant residuals throughout the home. Indeed, one hypothesis for traveler's associated Legionnaires' disease is that the individual is exposed to stagnant plumbing upon returning home (Verhoef et al., 2004). After prolonged absence, flushing should be considered as a preventive measure since stagnant water may have high concentrations of bacteria including *Legionella*, bad taste and odor, no disinfectant residual, and elevated concentrations of metals such as copper and lead.

Temperature Control. Elevating water heater temperatures is an obvious household intervention, though this can be restricted by building codes, which vary from state to state. For highly elevated water temperatures, the scalding risk may also not be worth the trade-off for certain elderly or less mobile individuals. Also, for many reasons delivery of hot-enough water temperatures to the point of use can be a problem in households just as it is for hospitals and hotels. Water in households typically sits

[8] See https://www.census.gov/programs-surveys/ahs/data/interactive/ahstablecreator.html.
[9] See http://amwater.com/corp/legionella-homeowners.

stagnant during the day, and homes can be vacant for long periods during vacations. As discussed previously, the choice of hot-water heater design is also important to minimizing the risk of legionellosis.

Distal Devices. *Legionella* can be amplified at distal sites such as faucets and showers in the household. Selecting faucets to minimize the potential for *Legionella* growth can be achieved by selecting simple designs without electronic activation and only with mixing valves if needed. If thermostatic mixing valves are justified for scald prevention, then models with the valve integrated to the body of the faucet with minimal volumes of tepid water would offer a lower risk (Charron et al., 2015).

Reverse osmosis units are not uncommon at the household level and can be installed as whole-house POU filters. Based on size exclusion, *Legionella* should be eliminated after passing through reverse osmosis. However, household filters, including reverse osmosis units and carbon black filters, can also remove disinfectants. Thus, if applied at the whole-house level, they could potentially leave downstream plumbing at risk of colonization. Carbon black filters, which are typically applied as faucet mounts or used in filters for water directly intended for drinking or cooking, provide surface area for microbial growth and result in elevated HPCs. A study by the World Health Organization indicated that there was no measureable human health risk associated with increased HPCs from POU filters (Hunter, 2003).

Disinfection. On-site disinfection is likely unrealistic for most homeowners although chlorination is commonly recommended to well owners for remedial purposes. Point-of-use UV units are gaining in popularity, for example, under sinks where drinking water is drawn and as part of refrigerator-dispensed drinking water. Such units need to be evaluated in terms of efficacy for *Legionella* control and most effective placement.

No national recommendations have been developed to help protect individual households from legionellosis. However, some studies have recommended steps to limit potential exposures to *Legionella* (e.g., Pedro-Botet et al., 2002). Much of the effort for homeowners focuses on water temperature and water flow during periods of decreased use. Increasing the water temperature in households to 60°C (140°F) can help limit *Legionella* growth in home hot-water systems, but must be weighed individually against the risk of scalding and burns. Tankless, on-demand hot-water heaters may provide an opportunity to limit the amount of water that is at risk and may have higher disinfectant residuals (Brazeau and Edwards, 2013b). Prevention of water stagnation while residents are not at home, such as flushing the taps at least weekly, may help to prevent *Legionella* growth. Alternatively, homeowners can decrease water-heater temperatures to levels that do not promote *Legionella* growth when they expect to be away from home for prolonged periods; this may be less effective in areas where normal ambient summer temperatures are high. For immunocompromised or high-risk individuals, additional measures such as POU filters for sinks and showerheads can be considered (Baron et al., 2014). Use of humidifiers, particularly those using water misting, should be discouraged among higher-risk patients (Hines et al., 2014; Yiallouros et al., 2013).

Cooling Towers

Heating, ventilation, and air conditioning (HVAC) systems are designed to condition and to distribute air to provide a comfortable indoor environment. HVAC systems can be a source of *Legionella* infections because they have abundant water and can disseminate *Legionella*-contaminated aerosols (Aaron, 2017). Within HVAC systems the two most likely sites to harbor *Legionella* are the humidification

and the cooling equipment. This section deals exclusively with cooling equipment, while the following section deals with humidifiers.

Evaporative heat transfer devices such as cooling towers and evaporative condensers are used to dissipate waste heat from the condenser of chillers providing air conditioning to a building. There are two basic types of evaporative heat transfer devices—a direct-contact device that exposes water directly to the cooling atmosphere, and a closed-circuit device that involves indirect contact between the heated fluid and the atmosphere. Their construction and operation are extensively detailed in documents by various organizations (such as the Cooling Technology Institute; ASHRAE, 2016).

Open and closed recirculating wet and wet/dry cooling towers may show some emissions because of drift and volatilization. Plume formation can be important in open and closed wet cooling towers when air with a high moisture content leaves the cooling tower, mixes with the atmosphere and begins to cool down. Both wet and wet/dry device types can be sources of *Legionella* infections due to their large use of water, their operating temperature, and their capacity to generate aerosols.

The single most important component of a cooling tower is the fill or heat-transfer surface, as different geometries and fill materials affect the heat rejection rate. Fills are susceptible to fouling, scaling, and microbiological growth (DOE, 2011). Within the water distribution and mechanical components of cooling towers, polypropylene, acrylonitrile butadiene styrene, and fiberglass-filled nylon have largely supplanted the bronze nozzles of earlier cooling towers, and PVC and fiberglass piping have replaced most iron and steel piping. Therefore, the materials typically used now are resistant to corrosion, erosion, and microbial growth (SPX, 2009).

Cooling towers are usually situated outdoors and open to the elements. This location makes them popular for birds and bugs to live in or around and susceptible to dirt and debris carried by the wind, providing nutrient sources for microorganisms in the system (DOE, 2011). A variety of microorganisms can grow in cooling towers during the course of normal operation, which involves water temperatures ranging from 29°C to 35°C (ASHRAE, 2000). Bacteria can grow in condensers and in the cooling tower fill, while algae can grow on wet cooling tower components exposed to sunlight. Biofilms are frequently found in chiller bundles, on the surfaces of heat exchangers, and in the system's piping (DOE, 2011).

By design, cooling towers use a significant amount of water, as they dissipate heat by evaporation. Geographic and climate concerns such as water availability or sewer usage restrictions may dictate unusually elevated water recirculation needs for the heat rejection equipment. However, the increased cycles can increase the concentrations of metals, minerals and contaminants (SPX, 2009).

Control Options

Control options in cooling towers are somewhat limited and based primarily on the use of disinfectants to prevent microbial growth. Materials selection during cooling tower design and construction can also affect whether the tower becomes a site of *Legionella* amplification. A major preventive strategy when cooling towers are not in use is to recirculate the water. Finally, though not traditionally considered as a control for cooling towers, future cooling tower designs using elevated temperatures could aid *Legionella* prevention.

Disinfection. Chlorination and hyperchlorination are commonly used chemical treatments to limit microbial growth in cooling towers, although numerous chemical disinfection methods have been used (Kim et al., 2002). However, these treatments generally do not completely eliminate the *Legionella*. If the treatments are discontinued, recolonization can occur after a lag period sometimes as short as two weeks. For example, Iervolino et al. (2017) showed the recolonization by *Legionella* of hyperchlorinated

cooling towers can take place within weeks or months of the initial treatment. Paranjape et al. (2019) found that continuous chlorine application in a cooling tower reduced microbial diversity and promoted the presence of *Pseudomonas,* creating a non-permissive environment for *Legionella* spp.

Silver and copper ions have also been used in cooling towers to control bacterial growth (Lin et al., 2002). In a study by Martinez et al. (2004), a chlorine concentration of 0.3 parts per million (ppm or mg/L) was combined with 200 parts per billion (ppb) of silver (Ag) and 1.2 ppm of copper (Cu). This method had an appreciable impact on levels of coliform bacteria, iron-related bacteria, sulfate-reducing bacteria and slime-forming bacteria in a cooling tower.

Constant use of a single biocide can promote the establishment of a treatment-resistant microbial community in the cooling system. The typical solution for this problem is to routinely alternate between two or more biocides. However, the use and handling of toxic biocides should be evaluated to prevent overexposure of the maintenance workers and the building occupants.

Chemical-free water sterilization methods such as ozone and UV light have also been used sporadically in cooling towers. Ozone is considered to be effective against microbial contamination at a concentration of 0.2 to 1.0 mg/L. However, ozone gas is harmful to humans and must be handled carefully to avoid human overexposure. Furthermore, incorrect implementation can hamper the smooth operation of the cooling system. Of the half million or more cooling towers in the United States, it is estimated that only 300 to 1,000 use ozone. Likewise, UV has not been widely accepted for cooling tower use because of scaling of the UV system and issues arising from improper application of the technology (Rossman, 2003). The efficiency of these technologies either by themselves or in combination with other water treatments remains to be proven.

Cooling Tower Materials. An important element in controlling biofilm growth within cooling towers are the materials selected for the construction of the heat rejection equipment. In reality, the equipment purchase specifications are mostly concerned with the performance and economics of the cooling tower operation. A study by Türetgen and Cotuk (2007) found that heterotrophic plate counts and *L. pneumophila* concentrations on galvanized steel were significantly higher than on six other construction materials used in a cooling tower (i.e., copper, stainless steel, polyvinyl chloride, polyethylene, polypropylene, glass). Corrosion-proof and anti-microbial resins are now being used for cooling towers (Sullivan, 2018). In selecting materials for cooling towers, not only is the bacterial growth potential of materials important, but also the performance and longevity of the materials in terms of how they are affected by the selected chemical and non-chemical treatments.

Temperature Control. Temperature control in cooling towers is not generally considered to be an option because the temperature rise in a condenser or heat exchanger will increase the potential for calcium carbonate scaling, which can damage the fill materials. Indeed, most fill materials cannot be utilized in temperature applications above 49.9°C to 51.7°C (120°F to 125°F). However, given the proven efficacy of raising potable hot-water temperatures to 60°C (140°F) to control *Legionella*, the Committee suggests that refrigeration, HVAC, and cooling tower manufacturers collectively design and develop new systems that can operate at condenser water temperatures whereby the temperature going to the cooling tower will be greater than 60°C. In this proposed conceptual system, the condenser water temperature coming from the refrigeration equipment or chiller would be 65°C to 70°C and travel first to a reheat heat exchanger. By heating the reheat water in the heat exchanger, the water temperature would drop to 60°C before transport to the cooling tower, dropping the temperature to 55°C and then back to the refrigeration equipment or chiller. At such operating temperatures *Legionella* would be unlikely to survive.

Such designs would require additional energy consumption to increase the corresponding refrigerant gas pressures and temperatures to heat the condenser water to such levels. However, additional heat,

in excess of the temperatures required, could be removed by a heat exchanger and used for the building's reheat water system, increasing the overall efficiency. Finally, the creation of cooling towers that could withstand such temperature increases could potentially reduce the need for chemical biocides.

Humidification Equipment

In both residential and commercial buildings, humidification equipment uses water to cool and humidify the air. These units come in two basic types. Isothermal units such as steam humidifiers use energy to produce a steam vapor and are considered non-aerosol-generating. On the other hand, adiabatic units allow direct contact between the water and the airstream, producing aerosols. Certain adiabatic units, such as atomizers or spray humidifiers, introduce water droplets directly into the airstream. Other adiabatic units, such as evaporative units and air washers, are considered non-aerosol-generating because the process only involves air absorbing the moisture as it passes over a pan or wetted device (ASHRAE, 2016).

Different designs of humidifiers have different levels of risk for *Legionella* growth (BMEC, 2009). Steam releasing-type humidifiers convert water to vapor that is then discharged into the selected space. Because of the high temperatures involved, and the fact that water droplets are not generated, this design is not considered a high risk for *Legionella* growth. Vaporization devices or direct evaporative coolers use a porous substrate to provide an extended surface area for water evaporation. The water is either circulated over the media or the media are rotated through a water bath. Thus, no water droplets are produced that could be contaminated with *Legionella* bacteria. The water used tends to be maintained at temperatures below the *Legionella* growth temperature range of 25°C to 43°C.

On the other hand, water spray devices such as misters, air washers and spray humidifiers can produce aerosols through the use of ultrasonic vibrators, spinning disks, or spray nozzles. When their source water comes directly from the building's cold-water supply or if the source water has been sent through reverse osmosis, these humidifiers can be used safely. However, when the source water is in holding tanks or in the pipes exposed to heat, the temperature of the water can reach 25°C to 43°C, a range that supports *Legionella* growth. Ultrasonic humidifiers and centrifugal sprays are thought to be most susceptible to *Legionella* contamination (BMEC, 2009). To limit the risk of legionellosis, these devices should be avoided for use in new buildings. Existing units of these types are recommended to be replaced during building renovation projects (PWGSC, 2013).

Regarding portable humidifiers, a review of the literature indicates that most of the disease transmission associated with these units is due to aerosol producing humidifiers, i.e., ultrasonic and impeller units. Generally, the disease transmission is because the humidifiers were not properly cleaned or disinfected (Public Health Ontario, 2017). The appropriateness of allowing bedside humidifiers in institutions housing patients and residents who are more vulnerable to respiratory disease has been a topic of considerable debate.

Control Options

Disinfection. There are limited chemical treatment options to reduce or eliminate *Legionella* bacteria from humidification systems. This is because the chemicals have the potential to be discharged into

HVAC air distribution systems and ultimately be inhaled by the building's occupants. Water treatments such as softening and demineralization address the quality of the supply water necessary for the operation of the equipment but not the potential for microbial growth. Several Korean studies found that the use of disinfectant chemicals directly in the water of personal humidifiers has caused interstitial lung disease in children (Park et al., 2014, 2017; Pickering, 2014). Other water treatments, such as the use of UV or photochemical ozone generators instead of chemicals, have been considered (ASHRAE, 2000). Regular monitoring is needed to determine whether these treatments remain effective (HSE HSG 274, 2013c).

Temperature Control. Water storage temperatures for all HVAC equipment are recommended to be either above, or below, the 25° to 43°C range where *Legionella* thrives.

Hydraulic Management. Rigorous maintenance of humidification equipment is critical including regularly scheduled maintenance of the system, avoidance of water stagnation in the water tanks, pans, and basins, and use of water treatment where necessary. If these precautions are not feasible, the equipment must be taken out of service. Similarly, for smaller humidifier units (portable or home size), rigorous maintenance and drainage are recommended as well as appropriate cleaning and disinfection offline with suitable agents as per the manufacturer's instructions (Public Health Ontario, 2017).

Hot Tubs and Swimming Pools

Legionella outbreaks have been caused by contaminated hot tubs (Benkel et al., 2000; Campese et al., 2010; Moore et al., 2015). Indeed, hot tubs were the third leading cause of legionellosis outbreaks among 27 investigations reported between 2000 and 2014, following potable water and cooling towers (Garrison et al., 2016). The warm water in these devices is often at the optimal growth temperature for *Legionella* growth (30°C to 40°C). Aerosols created by the water jets in some hot tubs can transmit the bacteria to people sitting in the units who are breathing very close to the water surface (Moore et al., 2015). Moreover, aerosols released from the water can be dispersed by air currents or ventilation systems, placing people outside the hot tub at risk for *Legionella* infection.

Hot-tub water is typically filtered and treated with chlorine, bromine, or ozone (Leoni et al., 2018). Although disinfection is the primary management option, the warm temperatures in hot tubs make it hard to maintain disinfectants at the levels needed to kill bacteria including *Legionella*. Therefore, hot tubs should be periodically inspected by health officials to ensure they are operating properly and adequately cleaned. Facility managers should check the amount of disinfectant in the water and the pH and have a regular schedule for cleaning that includes removing any films or algae from the sides of the hot tub. Filters in these units should be replaced in accordance with the manufacturer's specifications. If *Legionella* is detected in a hot tub, the facility manager should follow CDC[10] or American Society for Heating, Refrigerating, and Air-Conditioning Engineers guidelines for cleaning and disinfection (ASHRAE, 2015).

Although most recreational water *Legionella* outbreaks are linked to the warm water of hot tubs, the CDC also outlines guidance for pool operators. These include a 12-step program for prevention of recreational water illnesses, training, procedures for pool operations, and videos and guidance for the safe handling of pool chemicals.[11] Facility operators should know and obey all applicable laws and regulations. If there are shower facilities associated with pools, facility managers should be cleaning and disinfecting the showerheads and faucets on a regular basis.

[10] See http://www.cdc.gov/Legionella/downloads/hot-tub-disinfection.pdf.
[11] See https://www.cdc.gov/healthywater/ swimming/aquatics-professionals/index.htm.

EMERGING OPPORTUNITIES AND UNINTENDED CONSEQUENCES

There is much still to be learned about *Legionella* ecology and its response to engineering controls. Currently, knowledge of built environment microbiomes is rapidly expanding (NASEM, 2017), largely driven by next-generation DNA sequencing, which promises to provide new insights. At the same time, U.S. infrastructure is aging beyond its intended lifespan and experiencing shifts in water demand, along with changes in behaviors and expectations of water consumers. Thus, the current situation presents both opportunities and challenges.

Presently there is a major push towards advancing "green" building features in the United States, with the important goals of conserving energy, water, and materials. Water conservation features are driven by the need to reduce unsustainable water extraction, particularly as supplies experience greater pressure as a result of drought and other consequences of climate change. The need to reduce dependency on fossil fuels and limit production of greenhouse gases drives incorporation of energy-saving features, but these measures often have consequences for water systems as well. The U.S. Green Building Council (USGBC) reported that green building construction expenditures currently outpace those of general construction, with projected outlays of $224.4 billion in 2018 (USGBC, 2015). Developed by the USGBC, the Leadership in Environmental Engineering Design (LEED) certification system ascribes points for various building attributes, with "platinum" being the highest level of certification. Particularly relevant to this report are features by which such buildings can earn points for "potable water savings" and "energy savings" (USGBC 2016a,b). The following examples illustrate some of the complex and untested scenarios that can have unintended consequences and increase risk of *Legionella* growth in building water systems (Rhoads et al., 2015b) and highlight emerging opportunities to advance science and better understand and address such challenges.

Prebiotic and Probiotic Control of *Legionella*

Given that it is impossible to eradicate microbes or biofilms from engineered water systems, the possibility of intentionally shaping the *kinds* of microbes that colonize piped water systems to suppress pathogen growth niches has been proposed (Wang et al., 2013a). By definition, a *probiotic* approach would be to intentionally add such beneficial microorganisms, whereas a *prebiotic* approach controls the environment (e.g., water chemistry, pipe material, temperature) to favor desirable microorganisms. This exploratory concept remains to be tested and demonstrated in practice. Nonetheless, this is an interesting area for future research. As described in Chapter 2, there are several unique aspects of *Legionella*'s microbial ecology that lend support to the possibility of prebiotic or probiotic control.

One prebiotic approach extends from the examples of general biofilm control via nutrient reduction previously described in this chapter. Biological treatment that reduces the levels of biodegradable organic matter can help reduce the density of biofilm bacteria, and thus decrease the number of protozoan hosts available for *Legionell*a replication. In particular, the composition of organic matter could be tailored to select for a biofilm community that is a poor food source for amoebae (Amaro et al., 2015) or for protozoa that digest *Legionella* (Amaro et al., 2015; Anderson et al., 2011; Maita et al., 2018). This possibility is supported by the fact that certain free-living amoebae are known to preferentially prey on certain bacteria rather than *Legionella* (Shaheen and Ashbolt, 2019).

Alternatively, the thermal and disinfection controls described above may indirectly control *Legionella* by decreasing the population of free-living protozoa. Likewise, by manipulating other environmental factors such as oxygen levels, metals, organic carbon, stagnation, pipe materials, and other physicochemical and biological parameters, the ecology and life stage of free-living amoebae in water

systems (and hence *Legionella*) could be managed. Another possibility would be to impose conditions (e.g., through nutrient deprivation, disinfection, or temperature shock) that shift free-living amoebae populations to the cyst stage, hence reducing *Legionella* growth potential because it is only capable of growing in trophozoites, not cysts.

One probiotic approach entails adding microbes that are a more preferred food-source for amoebae than *Legionella* but are non-digestible. Since the amoebae would derive little nutritional benefit from grazing on such a biofilm, their populations would decline or encyst. In particular, water systems would be supplemented with microbes that compete with α-Proteobacteria, key prey for protozoan hosts of *L. pneumophila* (van der Kooij et al., 2018). Further, manipulating the types of free-living protozoa inhabiting the system presents several possibilities. For example, some amoebae are capable of digesting *L. pneumophila* (Amaro et al., 2015; Maita et al., 2018) or contain symbionts that do not allow ingested *Legionella* to replicate within the host (Maita et al., 2018; Okubo et al., 2018).

Prebiotic and probiotic approaches may be particularly attractive in the future given inherent limitations in existing engineered controls. In fact, relative resistance to both disinfectants and heat treatment are common features among *Legionella* and other pathogens that plague premise plumbing (Falkinham, 2015). As noted in Chapter 2, after intracellular replication in free-living protozoa, *L. pneumophila* can actually become more resilient to heat, oxidants, acids, osmotic pressure (Kwaik et al., 1997), biocides (Barker et al., 1992, Berk et al., 1998), and antibiotics (Barker et al., 1995, Garduño et al., 2002). Moreover, resistance to chlorine can spread among *L. pneumophila* on the ICE-box mobile genetic element, providing a mechanism for emergence of strains that persist in treated water (Flynn and Swanson, 2014). Thus, traditional use of disinfectants, depending on how effectively they are applied, may beneficially or detrimentally tip the microbial community balance towards one that favors *Legionella*. Better understanding the life stages and the ecology of free-living protozoa and *Legionella* in water systems could be critically important to advancing the possibility of prebiotic and probiotic control, as well as informing optimization of other more traditional engineered controls.

Unintended Consequences of Water Conservation

LEED-certified green buildings typically conserve 20 to 50 percent of potable water, although that value will rise as "off-grid" operations are adopted. To achieve water conservation goals, alternative sources of water are used for various purposes, including toilet flushing, landscaping, or even potable applications. Alternative sources include reclaimed water, greywater, and rainwater, which may present unique risks compared to traditional potable water. The other main approach to water conservation is incorporation of fixtures and appliances that use less water, such as low-flush toilets and low-flow and metered faucets. While current LEED certification does take into consideration "indoor environmental quality," the focus is on criteria such as ventilation, thermal comfort, daylight, tobacco smoke, and avoiding volatile organic compound-emitting materials, rather than water quality or *Legionella*. The need for these additional criteria is beginning to be recognized and would enhance the benefits of the green building movement (Cedeno-Laurent et al., 2018).

High Water Age

Deteriorating water quality due to high water age is a fundamental challenge of water storage, which many hospitals and other buildings require to ensure water security in emergency situations. For

example, the Centers for Medicare & Medicaid Services (CMS) has mandated that hospitals be self-sufficient for 96 hours without essential utilities and deliverable items, including potable water. Many hospitals elected to maintain large stocks of potable water to meet the required 96-hour reserve. Any efforts to conserve water inherently increase stagnation and overall water age, both at the municipal level (i.e., main distribution system water age) and at the building-level (i.e., premise plumbing water age) (Rhoads et al., 2016a). One survey found the premise plumbing water age in a typical LEED-certified healthcare suite to be eight days; it was more than six months in an off-grid office suite (Rhoads et al., 2016a). High water age has long been known to be detrimental to main distribution systems due to enhanced corrosion, development of taste and odor issues, loss of disinfectant residual, and regrowth of microorganisms (EPA, 2002b). Increased distribution system water age can also increase water corrosivity for premise plumbing (Masters et al., 2015). A national survey indicated that there is excessive "overdesign" of water mains based on actual fixtures and flow rates (Buchberger et al., 2015), which further exacerbates water age problems at the community scale.

Once water enters the complex, high surface area and warm premise plumbing environment, such problems are only magnified. Meanwhile, the ability to compensate for lower flows is constrained by current building codes, such as mandating larger pipe sizes (Klein, 2018). A study of a newly constructed residences with green plumbing features occupied by college students noted a clear pattern of diminished water quality at the least frequently used taps (Salehi et al., 2018). In the LEED-certified healthcare suite noted above, disinfectant residual was entirely absent at all sampling points; more than 80 minutes of flushing was required before the municipal chloramine residual could be detected (Rhoads et al., 2016a). Further, the plumbing materials themselves enhanced disinfectant decay, with chloramine decay rates being 20 to 144 times faster when the well-flushed water sat in the plumbing compared to in a clean glass container. As water stagnates, it is also more often within an optimal temperature range for *Legionella* growth. In the LEED-certified healthcare suite, *Legionella* spp. gene copies were nondetectable in the incoming water supply, but were in the range of 10,000 to 100,000 GC/mL in three of the five premise plumbing sampling locations (Rhoads et al., 2016a).

Low-Flow and Metered Faucets

Lower-flow fixtures, including toilets, dishwashers, washing machines, showerheads, and faucets are required by the EPA WaterSense program to reduce flows by at least 20 percent (EPA, 2016b). Lower flows reduce the rate at which consumers can draw water, but this can backfire because more flushing time is needed to obtain the target hot or cold temperature, depending on the application. Lower flow also pushes hydraulics into the laminar flow range, which is less effective for scouring biofilms, and can increase numbers of biofilm-associated *Legionella* (Liu et al., 2006). Metered faucets are very common in green buildings, only delivering a pre-determined aliquot and aiming to conserve water by incorporating electronic sensors to ensure that they are only opened when in use. Additionally, although such "hands-free" devices are intended to reduce spread of germs, ironically several studies have now confirmed that they have a propensity to grow *Legionella* and other pathogens, such as *P. aeruginosa* (Yapicioglu et al., 2011). Notably, Sydnor et al. (2012) cultured *Legionella* from 19 of 20 electronic faucets and only nine of 20 manual faucets co-located across three hospital units; this trend was even stronger when repeated sampling was taken into account. Further, *Legionella* colonizing electronic faucets were less responsive to chlorine dioxide disinfection than were *Legionella* in traditional faucets. Although it is not fully known why, the internal plastic components and the mixing of water create an ideal temperature for *Legionella* growth, which likely contributes to this problem.

Rainwater Harvesting

Collection of rainwater in cisterns is common throughout many parts of the world, particularly the rural tropics and sub-tropics, but this practice is also becoming a more intentional aspect of modern green building design elsewhere. The EPA does not regulate the water quality of residential rainwater harvesting systems, but some state and local agencies do issue voluntary water quality guidelines for residential rainwater harvesting systems. Yet, natural rainwater is not as "clean" as one might assume, as it is highly susceptible to atmospheric and rooftop sources of contamination, including bird droppings, heavy metals (Förster, 1999; Lee et al., 2010), herbicides (Bucheli et al., 1998), and pesticides (Zorbrist, 2000). The type of roof material also affects microbial water quality (Clark et al., 2019). A recent qPCR survey of *Legionella* in harvested rainwater tanks in Queensland, Australia encountered *Legionella* spp. in nearly 100 percent of tanks and *L. pneumophila* in 17 percent of tanks (Hamilton et al., 2017). A follow-up study in Philadelphia similarly noted qPCR detection of *Legionella* spp. in more than 50 percent of rooftop rainwater harvesting barrels (Hamilton et al., 2018b). Similar to findings from sediments in drinking water reservoirs (Lu et al., 2015), soil and dust are likely sources of these legionellae and associated protozoa.

Various factors associated with rainwater storage, collection, and use are likely to exacerbate potential problems with *Legionella*. Rainwater is characteristically low in pH and alkalinity, resulting in corrosive water whose problems were noted previously. Metal tanks are among the most frequently encountered materials (Hamilton et al. 2017) and will be directly affected by corrosion. Further, rainwater harvesting inherently entails storage, during which time typical water age problems are incurred and can be exacerbated by poor maintenance. Hamilton et al. (2017) noted in their survey of Australian tanks that 50 percent were never cleaned or desludged in their lifetime. Finally, the water savings incurred by rainwater harvesting can indirectly increase the water age within potable water plumbing. One study found that using rainwater to flush toilets resulted in a 58 percent to 80 percent reduction in potable water use, with premise plumbing water age at some taps exceeding three weeks (Nguyen et al., 2012).

Off-Grid Systems

At the extreme end of "green infrastructure" are off-grid or "net zero" buildings, which do not rely on an external water network for potable water or wastewater services (EPA, 2013). Such independence from water utilities is a primary goal of certifications such as the Living Building Challenge. The characteristics of these buildings include use of water-saving devices to reduce water consumption,[12] rain-water harvesting, cisterns, on-site grey water or black water reuse, constructed wetlands, composting toilets, xeriscaping, and local aquifer recharge among other practices (Rhoads et al., 2015b). Such design configurations, however, raise a unique set of challenges and corresponding public health concerns. It is critical that these water systems be managed to control risks from *Legionella* and other water-related pathogens.

A recent survey estimated the premise plumbing water age of an off-grid "net zero" building to be between two to almost seven months, far exceeding that of a conventional building (Rhoads et al., 2016a). A 3,000-gallon tank for storing roof-top-harvested rainwater plus supplemented groundwater was primarily responsible for such a high water age. Disinfectant was not added to the water; rather, the water was subjected to serial filtration to 5 μm followed by a granular activated carbon (GAC) filter and UV disinfection. *Legionella* spp. gene markers measured by qPCR were detected throughout the system, including immediately post-treatment, in the storage tank, and in hot and cold flushed and stagnant water at 10^3 to 3 x 10^4 GC/mL (Rhoads et al., 2016a).

[12] See https://living-future.org/lbc.

Because of the unique designs for off-grid buildings, each should have its own water management plan following the principles outlined in Chapter 5. Source water should be properly filtered and disinfected, considering that even in rainwater samples Rhoads et al. (2015) reported *Legionella*, as measured by qPCR. The potential for extended water age means that the water management plans should address recirculation of water within the building plumbing system.

As part of the water management plan, off-grid buildings should pay close attention to keeping the hot water hot (55°C to 60°C) and the cold water cold (less than 25°C). Use of heat pumps or solar hot-water heating may result in water temperatures that are insufficient to prevent *Legionella* growth (Rhoads et al., 2016). Temperatures will fluctuate on a diurnal basis and be influenced by seasonal and weather patterns if a solar heating system is not also paired with a non-solar water heater (van Amerongen et al., 2013). A review of the literature by van Amerongen et al. (2013) did not find, however, that solar heaters were more prone to *Legionella* detection than conventional heating systems, but they did point out that design and maintenance were important.

Flushing water lines and cleaning and inspecting storage tanks are important activities for off-grid systems, just as they are for public water systems; both should be included as part of the overall water management plan. Corrosivity of rainwater could put system components at risk and enhance conditions for *Legionella*.

Biowalls

Biowalls are an example of a green building feature that is gaining popularity. These walls of plants maintained in the indoor environment are advertised as a natural "botanical filter" that improves indoor air quality, helps "reduce sick building syndrome," and saves energy by recycling internal air.[13] However, a scientific literature review did not indicate that such claims have been tested. The perpetually moist environment of the biowall, along with a rich soil inoculum, maintained within a warm building envelope, could create an ideal habitat for *Legionella* proliferation. Further, the intentional "filtering" of air through the biowall clearly creates the potential for aerosol formation and occupant exposure. Thus, biowalls meet several criteria of a building system of concern worthy of scrutiny for its potential to be a source of *Legionella* exposure. Accordingly, appropriate engineered controls should be considered.

Unintended Consequences of Energy Conservation

As noted above, elevated water temperature is a master variable for *Legionella* control in buildings. Incentives in green buildings that encourage lowering this temperature to achieve energy savings can create conditions conducive to *Legionella* growth (Brazeau and Edwards, 2013b). Water heating is the second largest consumer of energy in the home and, accordingly, the EPA ENERGY STAR® program recommends a lower water-heater setting of 48.8°C (120°F) (EPA, 2019). This and other similar policies are in need of critical evaluation. For example, at one point the California Energy Commission (CEC) required recirculation for hot-water lines longer than ten feet, under the assumption that this would reduce water usage by lowering the time needed to achieve target shower temperature (Brazeau and Edwards, 2011); however, head-to-head studies revealed substantial heat loss and failure to achieve target temperatures with recirculation (Brazeau and Edwards, 2013a). Current California plumbing code now requires insulation of hot-water lines to conserve heat, and recovery of heat from drains is also encouraged (CEC,

[13] See https://www.purdue.edu/biowall/.

2019). Thus, there is a need for comprehensive cost-benefit analyses of actual energy savings achieved with various types of heaters, temperature settings, and corresponding plumbing configurations versus their impacts on water quality known to present risk factors for *Legionella* proliferation (Brazeau and Edwards, 2011). Analysis is needed to ensure that energy savings goals are actually met, while factoring in important public health considerations.

One comprehensive hospital case study clearly illustrates the unintended consequences of implementing reduced water heater temperatures (Blanc et al., 2005). Following the implementation of energy conservation regulations, hospitals in Switzerland were required to lower their hot-water temperature from 65°C to 50°C. To minimize bacterial contamination of their hot-water plumbing, the Lausanne University Hospital first upgraded its hot-water plumbing by eliminating dead ends and improving flow patterns. A thermal-shock treatment was then conducted before implementing on-site disinfection in 1995. Two separate premise plumbing systems were treated with: (1) ozone with a residual of 0.3 mg/L and (2) copper-silver at 0.3 mg/L. After three years, the positivity for *Legionella* spp. remained high in ozone-treated networks (66 percent to 56 percent) and in copper-silver-treated systems (90 percent to 93 percent). Increasing the temperature to 65°C at the water heater decreased the bacterial occurrence back to acceptable levels, although some areas remained persistently positive and were associated with poor hot-water recirculation leading to temperature losses (Blanc et al., 2005).

The experience at the Lausanne University Hospital demonstrates the importance both of elevated tank temperatures and maintaining sufficiently hot delivery lines. Nonetheless, reducing the temperature at the water heater outlet and shutting down the recirculation during low-usage periods (e.g., night, weekends) remain two major targets of energy conservation. Well-documented case studies in real systems show clearly that a reduction in temperature at the water heater outlet can lead to a significant increase in the likelihood of *Legionella* detection and the level of contamination at the tap. Further, shutting down the recirculation during the night will create stagnant conditions for periods of eight hours or more. Even in insulated systems, water will reach the ideal temperature for *Legionella* growth during such long stagnation periods (Bédard et al., 2016a).

Energy conservation projects that add a heat exchanger to pre-heat the water prior to the water heater have also been increasing in popularity in healthcare facilities. The installation of these devices should be carefully studied to evaluate operating conditions. The very large surface present in heat exchangers, coupled with temperatures ranging between 25°C and 43°C, provide ideal conditions for *Legionella* growth. Recent field investigation revealed contamination of such a heat exchanger by a *L. pneumophila* strain that matched clinical isolates from cases occurring a few weeks after the installation of the device (Bédard et al., 2016b). Disinfecting the device on a weekly basis and determining operating conditions to minimize *L. pneumophila* should be mandatory in healthcare facilities.

Other options for reducing energy demand of water heating include solar heaters and on-demand heaters. Solar heaters come in various configurations, typically employing a pre-heat tank and taking advantage of water stratification to draw water from the top before either being used directly, feeding a traditional tank heater or on-demand water heater. This subsequently incurs less energy input to heat to the target temperature. A typical feature of solar water heaters is some level of added water storage, which takes advantage of the high heat capacity of water. Rhoads et al. (2016a) observed that the added storage incurred by a solar water heater in a net zero energy home increased the hot-water age from less than one day to between two to three days. Further, due to cloudy days, the solar pre-heat tank may essentially end up in the optimal temperature range for *Legionella* growth. *Legionella* spp. copy numbers measured by qPCR in the hot-water manifold that received the heated water and delivered it to taps were markedly high, upward of 10^6 GC/mL (Rhoads et al., 2016a). On the surface, on-demand heaters could be an effective alternative, only delivering hot water where and when needed, and these devices

are currently recommended by CEC (2019). However, there are many logistical constraints to their installation and use, and their benefits for *Legionella* control need to be more critically evaluated (Brazeau and Edwards, 2013c).

Potential Trade-Offs with Other Microbial Risks

Finally, it is important to consider whether recommendations herein intended for *Legionella* control could potentially have unintended consequences by favoring survival of other pathogens that are problematic in premise plumbing. A report sponsored by the Water Research Foundation (Project 4813) summarized common challenges encountered in premise plumbing that favor the proliferation of multiple pathogens, in particular *P. aeruginosa*, nontuberculous mycobacteria, *Acanthamoeba* spp. and *N. fowleri* (Pruden et al., 2013), though other examples include *Acinetobacter baumanii* and *Aeromonas* spp. Falkinham (2015) described several key commonalities among such organisms, including preference for biofilm environments, capacity to resist predation by protozoans, tolerance to disinfectants, and antibiotic resistance. Ideally, such commonalities could be capitalized upon to identify "silver bullet" approaches that offer protection against all pathogens that, like *Legionella*, are prone to proliferation in premise pluming. Indeed, efforts to reduce biofilms and amoebae hosts described in this chapter should in theory also address amoebal pathogens occurring in the plumbing. However, given that some of these organisms are markedly tolerant of disinfectants (e.g., mycobacteria), the higher doses required could pose other concerns, including generation of disinfection byproducts and selection of strains that are more tolerant of disinfectants. Also, whereas chloramines appear to be particularly effective against *Legionella* spp., *Mycobacterium avium* levels can increase when water systems are switched from chlorine to chloramine (Pryor et al., 2004; Wang et al., 2013b). Concerns have also been raised that drinking water disinfectants might inadvertently select for antibiotic-resistant bacteria, due to multifunctional or co-located antibiotic resistance genes, as was evidenced by a metagenomic-based DNA sequencing study (Shi et al., 2013). In particular, metal and antibiotic resistance traits are commonly co- or cross-selected among bacteria, begging the question of whether copper-silver ionization exerts similar effects when applied to drinking water (Chen et al., 2015). Khan et al. (2016) observed that chlorine resistance and minimum inhibitory concentration of various antibiotics positively correlated among several tap water bacterial isolates. Long-term exposure to low levels of chlorine was also recently associated with selection of antibiotic-resistant *P. aeruginosa* (Mao et al., 2018; Shrivastava et al., 2004) and upregulation of antibiotic resistance genes in *A. baumannii* (Karumathil et al., 2014).

Elevated water temperatures appear to also reduce growth of most pathogens in premise plumbing, but slightly hotter water temperatures may be necessary for mycobacteria. For example, viable mycobacteria have been observed in household water heaters, but numbers of positive heaters were substantially lower when the temperature was higher than 55°C (Falkinham, 2011). In the lab, 90 percent survival of mycobacteria was observed following exposure to 50°C for 60 minutes (Schulze-Röbbecke and Buchholtz, 1992).

Thus, there is need for research that harmonizes engineered control efforts to minimize the risk of other microbial problems, including growth, virulence, and antibiotic resistance of multiple pathogens. Ideally, selected controls for *Legionella* should have comprehensive benefits for control of other pathogens in water systems.

CONCLUSIONS AND RECOMMENDATIONS

For any given building water system, there are multiple strategies that can be successfully employed and should be used. Figure 4-5 provides an overview of the controls discussed in this chapter and the importance of considering their integration and applicability to various water systems. The different strategies available for controlling *Legionella* in water systems are feasible at different stages of a building's life cycle, with some being feasible mainly during initial construction (such as the choice of plumbing materials) while others are implemented during ongoing operation and maintenance (such as disinfection and flushing). Table 4-6 summarizes how the various control strategies should be considered at different stages of a building's life: design, commissioning, operations (including routine monitoring), and corrective action when necessary. It is critical to recognize that no single control strategy should be relied upon to control *Legionella* in building water systems, and multiple barriers are encouraged to the extent possible (Figure 4-5). Also, the effectiveness of many of the controls are interdependent, for example, optimal hydraulics are required for effective delivery of thermal and chemical disinfectant while reactivity of the plumbing materials and the water source chemistry could lead to disinfectant decay. Furthermore, as summarized in Table 4-1, not all controls are relevant to all water systems. For example, while thermal control is a primary barrier against *Legionella* in building systems, it cannot be applied to large engineered

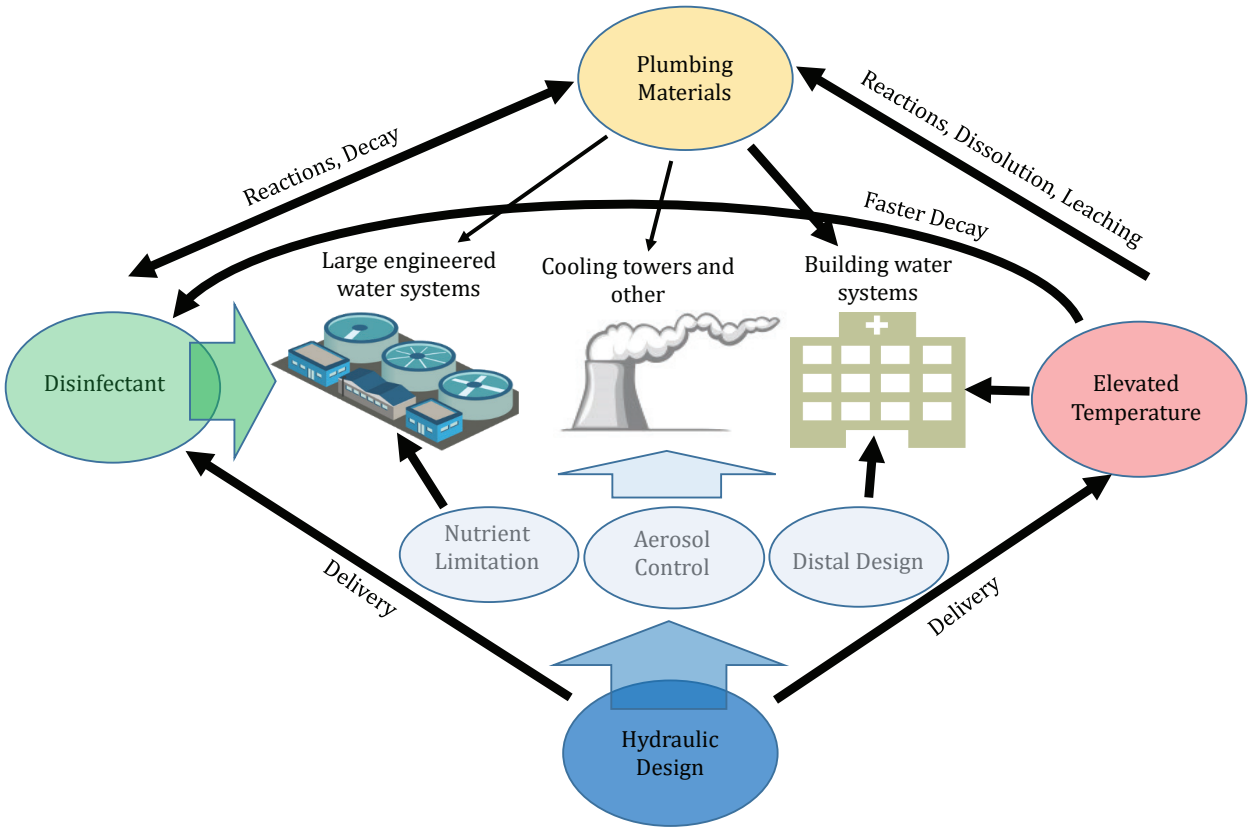

FIGURE 4-5 Interactions between *Legionella* controls in different water system types. Disinfection and hydraulic design apply to all systems. Only key examples are provided; not all systems or scenarios are represented.

TABLE 4-6 Summary of Implementation of Various Engineering Controls and Corrective Actions at Various Stages of Building Design, Commissioning, and Operations

Control	Design	Commissioning	Operations	Corrective Action
Hydraulic Control	pipe sizing and flow distribution to minimize water age and avoid stagnation	minimize periods of no use, preventive flushing during periods of no use	verify hydraulic balancing	modify hydraulics, correct deficient circulation
Materials Issues	select biostable materials and ensure corrosion control	cleaning, preventive flushing, disinfection	verify corrosion control	replace failing materials, eliminate corrosion byproducts
Source Water Quality	consider the quality of the source water: corrosivity, nutrient content, and ability to maintain disinfectant residual	delay filling of system during commissioning to avoid long periods without use	verify corrosion control, disinfectant residuals, and general microbiological water quality indicators	flush, adjust pH, hardness, corrosivity, etc. or switch to improved water supply
Temperature	minimize distance between hot-water heater and distal points, select hot-water heater, heat trace	delay start-up of hot-water system during commissioning to avoid long periods without use	maintain target temperatures across the system	adjust operational temperature
Disinfection	include chemical disinfection in design	apply shock disinfection	set disinfectant residual targets and monitor for compliance across the system	apply shock disinfection
Aerosol Transmission Prevention	select devices to minimize aerosol formation	verify absence of *Legionella* in premise plumbing before occupancy		replace devices to minimize aerosol formation
Water Management Plan	include from beginning of design	determine and apply specific commissioning plan for *Legionella* control	apply management plan	modify management plan

systems, such as wastewater treatment plants, because of the nature and scale of these systems. Other competing goals, such as commitment to water and energy savings for green building certification, must also be taken into consideration. Water management plans (discussed in detail in Chapter 5) are essential to *Legionella* control for any water system, as they provide the opportunity to adapt and tailor the strategy to the specific system of concern and employ and integrate all applicable barriers (see Table 4-6).

Two rows in Table 4-6 do not correspond precisely to controls discussed in this chapter. First, source water quality is listed (rather than the narrower nutrient limitation), as there are important water quality considerations at each stage of a building's life cycle and multiple control strategies will affect water quality. Second, there is a final row on water management plans for protecting a building from a *Legionella* outbreak because having a plan itself is a critical control. (Such plans are discussed in detail in Chapter 5.)

The conclusions and recommendations below highlight key lessons regarding *Legionella* control strategies for the building and device types discussed in this chapter.

For all types of buildings, hot-water heater temperature should be maintained above 60°C (140°F) and the hot-water temperature to distal points should exceed 55°C (131°F). Maintaining temperature outside *Legionella's* preferred growth range is the paramount *Legionella* control strategy for all buildings that provide hot water and has been proven successful by numerous longitudinal field studies. Temperature control is most effective in large, complex hot-water systems that are hydraulically balanced, with dead-end pipes removed and faulty devices that compromise the distribution of hot water identified and replaced.

There is growing evidence that, compared to free chlorine, a monochloramine residual better controls *Legionella* risk from building water systems, although the reasons for the improved performance are not yet clear. It is possible that amoebae trophozoites are more sensitive to monochloramine, causing the amoebae to encyst and thus preventing the proliferation of *Legionella* within their host. Additional research is needed to examine the precise action of monochloramine on *Legionella* persistence and growth within pipeline biofilms. Better understanding of the potential for nitrification in building plumbing is also required, as this reaction could negatively impact the effectiveness of a chloramine residual for *Legionella* management.

Additional research is needed to evaluate the potential for nutrient limitation (concentration and composition) to control *Legionella* growth in distribution and building water systems. These studies should examine, in full-scale drinking water systems, the impact of nutrient reduction on the concentration and composition of the microbiome in biofilms and water including amoebae growth and life stages and the subsequent effect on occurrence and decrease of pathogenic *Legionella* species.

New NSF/ANSI standards regarding microbial growth potential of materials are needed so that water utilities, plumbers, and building contractors can include *Legionella* control when making decisions about pipe material usage. Certain plastic components (e.g., PEX) tend to lead to bacterial proliferation. Iron components in distribution systems and premise plumbing should be replaced or otherwise managed with appropriate corrosion control to avoid disinfectant decay and release of iron as a nutrient for *Legionella*. Because of conflicting accounts in the literature about their role in *Legionella* growth, copper pipes cannot be relied on as a barrier to *Legionella* colonization and growth. More research is needed to identify circumstances when copper's antimicrobial properties are enhanced.

There is clear evidence of *Legionella* amplification in the distal parts of some hot-water systems, likely due to a combination of water stagnation and loss of temperature control and disinfectant residual. Some features of distal devices such as aerators, thermostatic mixing valves, complex designs, and shower hose materials have been linked to increased prevalence of *Legionella*. Additional research is needed to understand the conditions in distal reaches of premise plumbing that promote the amplification of *Legionella* so that improved distal devices can be designed.

Research is needed on new control technologies that limit the capacity of devices and building water systems to generate aerosols, particularly those smaller than 10 microns. The formation of aerosols is an important risk factor in the transmission of *Legionella*. Faucets and showerheads that limit the formation of fine mists should be used in locations where high-risk individuals could be exposed (e.g., hospitals). Technologies to minimize aerosols from cooling towers should strive for the highest efficiencies, and older cooling towers should be retrofitted with newer drift eliminators that meet higher standards.

Research is needed to better understand the persistence of distribution system disinfectant residuals within building plumbing. Public water supplies that maintain a disinfectant residual and manage hydraulics to prevent stagnation (such as through routine flushing and cleaning of storage tanks) are helping to reduce *Legionella* exposure from the distribution system. Nonetheless, it is unclear to what extent the disinfection residual can achieve *Legionella* control within premise plumbing, for both single-family homes and small buildings as well as larger buildings.

Guidance about *Legionella* is needed for homeowners, especially consumers from at-risk segments of the population. In particular, there is a need to identify plumbing configurations and devices that inadvertently increase risk of *Legionella* proliferation as well as accessible, practical control options such as flushing taps after periods of disuse. Residential water systems can benefit from most of the control strategies discussed in this chapter, yet they are almost never formally implemented because of a lack of understanding or awareness on the part of homeowners and occupants.

Low-flow fixtures should not be allowed in hospitals and long-term care facilities because of their high-risk occupant populations. Low-flow fixtures have been promoted to conserve water and in some cases energy. However, because of their lower flow, these fixtures, primarily low-flow faucets but also showers, increase water age and restrict disinfectant levels, including the disinfection provided by elevated water temperatures. As such, low-flow fixtures present a greater risk for *Legionella* development in the plumbing systems that feed them.

New designs are needed to help advance control of *Legionella* in cooling towers and humidifiers. Humidifier designs that produce water droplets within the temperature range conducive to *Legionella* spp. growth (such as evaporative pan, drum-type, water spray-type, sprayed coil-type humidifiers or air washers) should be avoided for use in new buildings, and existing units of these types should be replaced during building renovations. When designing and locating HVAC systems, it is important to prevent *Legionella* contamination and growth by considering equipment and material selection, proper drainage, and access for maintenance. Strategies relying on disinfectants should consider using alternate types of biocides at regular intervals, since bacteria can regrow in cooling towers when biocide use is infrequent and irregular. Finally, cooling tower manufacturers should collectively design new systems that can operate at condenser water temperatures whereby the temperature going to the cooling tower will be greater than 60°C.

Green buildings have exacerbated many of the problems with *Legionella* by lengthening water residence times (which leads to loss of disinfectant residual) and lowering hot-water temperatures in premise plumbing. Criteria for certifying green buildings, energy-conserving features, and water-conserving features should be modified to take into account risk factors for growth of *Legionella* and other water-based pathogens in building water systems. Substantial water conservation can still be potentially achieved while protecting public health with more overt management of water age, e.g., through routine flushing of a target fraction of the water use. Given the strong evidence that water heater settings below 60°C place a system at risk for *Legionella* growth, appropriate plumbing designs to conserve heat in the system may be the only reasonable path forward.

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5
Regulations and Guidelines on *Legionella* Control in Water Systems

In the United States, management of *Legionella* in water systems occurs on an ad hoc basis, spanning from regulations that require some buildings to have water management plans that include monitoring of water samples for *Legionella* along with treatment, to no requirements at all. In between exists a range of codes, standards, and guidance documents that have been sporadically adopted and typically target some of the high-risk zones for *Legionella* growth. Contributing to this widespread inconsistency in approaches to managing *Legionella* is the lack of any federal law that targets *Legionella* contamination of water supplies and building water systems as sources to be controlled.

This chapter begins by describing why the Safe Drinking Water Act does not provide any substantial control of *Legionella* in water systems. It then describes the many regulations, directives, codes, and guidance documents that can affect whether *Legionella* management occurs in the United States and the resulting significant lack of coverage. The chapter also describes the approach Europe and other countries have taken to manage *Legionella,* where stricter regulations have been imposed, and discusses how effective the regulations have been to date. For both the national and international regulations or guidance, this chapter describes the regulation, the control methods advocated, whether there is a *Legionella* monitoring requirement (and if so, whether it is based on percentage positive or concentration), and demonstrated effects of the guidance or regulations on Legionnaires' disease rates or results of environmental sampling for *Legionella*. Finally, the chapter's conclusions and recommendations suggest how the universe of approaches in the United States can be improved upon to better protect the public from exposure to *Legionella*.

LACK OF FEDERAL LAWS AND REGULATIONS PERTINENT TO *LEGIONELLA*

The Safe Drinking Water Act (SDWA) was originally passed by the U.S. Congress in 1974 to protect public health by regulating the nation's public drinking water supplies. The law was amended in 1986 and 1996 and requires actions by the U.S. Environmental Protection Agency (EPA) to protect drinking water and its sources—rivers, lakes, reservoirs, springs, and groundwater. (The SDWA does not regulate private wells or systems that serve fewer than 25 individuals.) Congress directed the EPA to address *Legionella* through the development of a treatment technique requirement, which is used when monitoring for the contaminant is deemed infeasible or unreliable. In 1989, EPA enacted the Surface Water Treatment Rule (SWTR), which requires public water systems using a surface water supply, or a groundwater

supply under the direct influence of surface water, to filter and disinfect the water (the latter of which is meant to control microbial contamination including *Legionella*). The SWTR requires disinfectant residual to be monitored in the distribution system and at the entry point to the distribution system. The disinfectant level must be at least 0.2 milligrams per liter (mg/L) at the point of entry and detectable in at least 95 percent of samples collected within the distribution system. Public water systems must maintain a residual disinfectant level of less than 4.0 mg/L as a running annual average within the distribution system, as outlined in the Stage 1 Disinfectant/Disinfection By-Product Rule. The SWTR established a Maximum Contaminant Level Goal (MCLG, a non-enforceable guideline) of zero *Legionella* organisms in drinking water. This scenario of having no *Legionella* present in a drinking water system is consistent with the Centers for Disease Control and Prevention's (CDC) position that there is no known safe level of *Legionella*.[1] However, potable water supplies are not sterile, and *Legionella* exists in distributed water at some non-zero frequency of detection (LeChevallier 2019a,b).

The SWTR has been effective for controlling enteric organisms, such as norovirus, *Giardia* and *Cryptosporidium,* using a multiple-barrier approach at the treatment plant. These pathogens are reduced via filtration and can be inactivated via disinfection given the correct disinfectant and contact time (e.g., *Cryptosporidium* is resistant to chlorination but can be inactivated with ozone or ultraviolet [UV] light). These enteric viruses and protozoa do not multiply in the distribution system or in premise plumbing. Unfortunately, these principles do no extend to *Legionella* and some other environmental pathogens, which can grow in the pipe network after treatment. Hence, even a few cells that enter the distribution system can seed plumbing downstream. As discussed in Chapter 4, municipal water systems are not thought to be a major source of *Legionella* because tap water suitable for potable consumption is disinfected and is usually below the optimum temperature for growth of *Legionella*. However, low levels of *Legionella* may break through treatment barriers when the microbes are entrapped in the cysts of free-living amoebae or inside protozoa hosts where they are protected from disinfection (Dupuy et al., 2011). *Legionella* can also grow in oligotrophic environments where the disinfectant residual has declined and biofilms have developed (LeChevallier, 2019b).

Although there are many reports of *Legionella* proliferation in building water systems (see Chapter 3), there are relatively few monitoring studies of the organism in the distribution systems of U.S. public water supplies. Wang et al. (2012) detected *Legionella* species (spp.) using quantitative polymerase chain reaction (qPCR) in two chloraminated drinking water distribution systems: *Legionella pneumophila* was detected in 5.6 percent of samples and *Legionella* spp. concentrations were reduced 45-fold after tap samples were flushed for three minutes (suggesting that the microbes were primarily in the distal lines of the sampling taps). Lu et al. (2016) examined large volume (90 L) ultrafiltration concentrates from six sites within a distribution system in Georgia and frequently (57 percent) detected *Legionella* spp. by qPCR at an average concentration of 85 cell equivalents per liter. *L. pneumophila* was detected at similar frequency (6 percent) as the previous study. Concentrations of *Legionella* spp. were 0.4- to 78-fold higher in the distal sections of the distribution system compared to the entry point, suggesting growth within the distribution system.

Some municipal drinking water systems have summertime water temperatures that are favorable for the growth of *Legionella*, especially in southern climates where water temperatures may be greater than 30°C. LeChevallier (2019a,b) detected culturable *L. pneumophila* using the Legiolert™ assay in 15 of 1,087 (1.4 percent) distribution system samples (after flushing the taps for three to five minutes), and all positives occurred when water temperatures were greater than 18°C. Concentrations of *L. pneumophila* were less than 100 MPN/L except when chlorine residuals were less than 0.1 mg/L. The studies concluded that it was important that water utilities maintain at least a 0.1 mg/L chlorine residual, particularly

[1] See https://www.cdc.gov/washington /testimony/2013/t20130205.htm.

when water temperatures are greater than 18°C. Riffard et al. (2001) detected *Legionella* by both culture and molecular methods in warm and cold groundwater, which under the EPA Groundwater Rule (Federal Register Volume 71, Number 216, 2006) may not be required to be disinfected. Many water supplies have storage tanks that may be prone to high water temperatures where water stratification can prevent mixing and cause subsequent loss of a disinfectant residual. Lu et al. (2015) detected *Legionella* spp. including *L. pneumophila*, *L. pneumophila* serogroup1 and *L. anisa* by qPCR in 66.7 percent of municipal drinking water storage tank sediments from 18 sites across the United States. At least one outbreak of Legionnaires' disease has been associated with a community water system storage tank whose chlorine residuals were low (Cohn et al., 2015).

Despite the water utilities' maintenance of a distribution system residual, the responsibility for most public water systems ends at the meter or property line; utilities have little control over how building owners maintain their premise plumbing systems. Thus, even the best water quality delivered by a public water supply can degrade once it enters a large building. Large building complexes may contain miles of internal plumbing with features much more favorable for bacterial growth than the main distribution system (NRC, 2006); thus, it is not reasonable to expect that minimal disinfectant residuals in the distribution system persist throughout the premise-plumbing network. Flushing specific devices (e.g., showerheads or faucets) may be practical (albeit time consuming) in such large buildings, but may be impractical for the entire building system and could negate water conservation practices. For small buildings and single-family residences, the plumbing network is much simpler. Although stagnation occurs, intervals of high water use (e.g., showering, bathing, washing clothes) will periodically bring a disinfectant residual into the building. Building owners and homeowners should be made aware of the practices that can reduce disinfectant levels and increase the risk of bacterial growth (such as whole-house filters and water softeners). Thus, a partnership between the building owners (or those maintaining the plumbing system) and the public water utility is vital. Such shared responsibility requires communication, coordination, and close consultation, which is lacking in most cases.

Ironically, the SDWA itself can be a barrier to improving water quality in some building systems. As an added measure to manage *Legionella* risk, hospitals and long-term care facilities are increasingly using on-site disinfectants. However, the addition of disinfectant to a water system serving 25 or more people deems the building a "consecutive water system" under the SDWA. This means that the building owner can be required to comply with all the requirements that apply to a public water system, including bacteriological monitoring, control of disinfection byproducts, corrosion, and water quality reports to consumers, among others—a substantial burden and cost to building owners. Some have claimed that such an interpretation of the SDWA would make implementation of building water treatment systems untenable, as there would be hundreds of thousands of water systems to regulate. For many systems, the disinfectant boost would be only on the hot-water system (where *Legionella* tends to proliferate), which some do not consider a potable supply. EPA, however, defines water "intended for human consumption" as water used for drinking, bathing, showering, hand washing, food preparation, dishwashing and maintaining oral hygiene (40 Code of Federal Regulations [CFR] §141.801 and 63 FR 41940, Aug. 5, 1998), which clearly encompasses hot-water systems.

A provision in the SWDA (40 CFR §141.29) allows states to combine consecutive systems for monitoring and compliance purposes. This provision could substantially reduce the transactional complexity of implementing treatment in building water systems. Yet EPA has provided no guidance on this provision, and there are no examples of any state using it. At the current time, buildings that have installed some type of secondary control for *Legionella* protection can technically be regulated as public water supplies; in practice, whether they are depends on the intensity of enforcement by state environmental agencies.

Given the lack of concrete requirements stemming from the SDWA and the limited jurisdiction of water utilities responsibility, it can be concluded that the SDWA is not protective of the end user with respect to *Legionella* contamination. Water leaving drinking water distribution systems is not intended to be nor is it sterile, and *Legionella* spp. are going to be found in building water systems if looked for (e.g., Donohue et al., 2019).

STATE AND LOCAL REGULATIONS AND OTHER ENFORCEABLE POLICIES

Despite the absence of federal regulations or laws that could broadly control the presence of *Legionella* in water systems, there are local and state regulations that attempt to do just that. Likewise, agencies of the federal government have certain enforceable policies that affect buildings under their control. This section discusses the policies of the U.S. Department of Veterans Affairs and the Centers for Medicare & Medicaid Services, as well as New York City and State regulations for *Legionella* control in cooling towers and in certain healthcare facilities. Plumbing and building codes can also significantly impact control of *Legionella* amplification and transmission in buildings and can be widely enforced, but only at certain times during the life of a building.

Department of Veterans Affairs Directive 1061

Veterans Health Administration (VHA) Department of Veterans Affairs Directive 1061 establishes policy for the prevention and control of healthcare-associated Legionnaires' disease in VHA-owned buildings in which patients, residents, or visitors stay overnight (DVA, 2014a). Included are 170 medical centers that provide acute care, 134 community living centers, and 48 domiciliaries. The 2014 policy is premised on the notion that healthcare-associated Legionnaires' disease is most likely caused by the building's water systems, particularly the hot-water system. VHA follows the CDC definitions for "definite" and "possible" healthcare-associated Legionnaires' disease described previously (see page 106). The 2014 Directive replaces VHA policies that took effect in 2008, 2009, and 2012.

The Directive outlines a comprehensive approach to *Legionella* management, similar to some of the U.S. guidance documents and standards discussed later in this chapter, as well as several European regulations. The VHA approach consists of assessing risks, monitoring water quality, and implementing commensurate engineering controls to limit the growth of *Legionella*. Also included is monitoring of implemented controls, validating that the control measures are effective at inhibiting *Legionella* growth, and modifying measures as necessary. The Directive relies primarily on temperature control and biocides to control *Legionella* amplification in building water systems.

The preamble to the Directive sets up the tradeoff between temperature control of *Legionella* and scalding. *Legionella* are killed above 50°C (124°F), but above 110°F people are at risk of scalding. Hence, the directive does not require 50°C to be maintained in the distal parts of the hot-water system. Although this tradeoff complicates *Legionella* control in the VHA system, it was thought necessary because of their elderly and vulnerable patient population. The cold-water system is not thought to support *Legionella* growth, but it could if piped water temperatures remain greater than 25°C for several weeks.

The VHA Directive is structured around the various responsibilities of many people within VHA who will implement the Directive. In particular, the director of every medical facility is to establish a multi-disciplinary Facility Water Safety Committee to be chaired by the medical facility associate director and to include representatives from engineering and facilities management, infectious diseases, infection prevention and control, pathology and laboratory medicine, hemodialysis (if performed on

site), safety and industrial hygiene, and occupational health. The facility directors must ensure that each building subject to the Directive has a written Legionnaires' disease prevention plan in compliance with the Directive. This plan must include:

a. Schematics of the building water systems (hot and cold) that show how water is distributed, circulated, stored, heated and cooled, treated, and monitored.
b. A risk assessment of the building for healthcare-associated Legionnaires' disease. This is an annual evaluation of factors that may indicate increased risk, such as patient population risk factors, presence of building units associated with increased risk (e.g., transplant units), past cases of healthcare-associated Legionnaires' disease, and past positive environmental test results.
c. Identification of water system management points for the building's potable water system(s), where monitoring and controls can be implemented to prevent the growth of *Legionella* and prevent scald injury.
d. Establishment of engineering control strategies including control limits, a monitoring schedule, and a dead-leg elimination and prevention plan.
e. Documentation of the water quality monitoring and monitoring of control measures, including process flow diagrams of the different control strategies and monitoring for each building's hot- and cold-water plumbing systems.
f. Validation that the control measures are effectively inhibiting *Legionella* growth, which involves monitoring for both *Legionella* and Legionnaires' disease.

The engineering controls discussed in the Directive are more expansive than in the 2009 VHA directive and rely primarily on temperature control supported by biocide use. The particular temperature requirements are that hot-water storage tanks must be maintained at a minimum of 140°F (60°C), instantaneous and semi-instantaneous heat exchangers must be at 130°F (54.4°C), and water in the potable hot-water system piping must be no lower than 124°F (51.1°C). To avoid scalding, the water at outlets must not exceed 110°F (43.3°C), so thermal mixing valves must be used (as discussed in Appendix B of the Directive). Biocide use is considered optional, but if used, monitoring of the residual is required in various locations. Their accompanying Plumbing Design Manual (DVA, 2014b) better explains all the engineering controls. When a case of Legionnaires' disease is found at a facility, then emergency remediation is needed, which may include thermal eradication or shock chlorination.

Validation of the control measures requires both monitoring for Legionnaires' disease and environmental monitoring of *Legionella*. The environmental monitoring consists of quarterly testing of *Legionella* in the hot- and cold-water systems (at least ten outlets each per building). The facility can choose to test additional areas and to take swab samples. If samples are positive, then additional testing of that outlet and nearby outlets and remediation are required. Depending on where positive hits occur, the entire building water system may require remediation. Notably, any amount of *Legionella* detected is considered positive and requires action (although *Legionella* concentrations are recorded).

Clinical testing of pneumonia patients at VHA facilities for Legionnaires' disease uses both the urine antigen test and culture methods, especially if there are environmental samples that test positive for *Legionella*. When a definite healthcare-associated case of Legionnaires' disease is found at a VHA facility, remediation of potentially implicated water systems is required followed by environmental testing to confirm remediation success. Possible healthcare-associated cases of Legionnaires' disease trigger a slightly less heightened response that may involve environmental testing and remediation. Investigation of environmental sources of definite or possible healthcare-associated cases can go beyond the building premise plumbing to other systems.

After publication of the Directive in 2014, to oversee the program implementation VHA staff began collecting clinical and environmental data and performed site visits as needed (Ambrose et al., 2019). Among many of the challenges observed was how difficult it is to maintain hot-water temperatures and biocide residuals at end points, particularly in large water storage tanks. It also became apparent that diagnosis of Legionnaires' disease relied primarily on use of only the urine antigen test instead of both the urine antigen test and clinical culture. They also noted a lack of habitual follow-up on optimizing implementation of engineering controls.

Despite these challenges and others, an analysis of the clinical data from the first three years of Directive 1061 implementation was recently published (Gamage et al., 2018). Almost 50,000 urine antigen tests were performed for VHA patients during this time period. The highest percentage of positives for the urine antigen tests were in the summer months and in the Northeast. Of the total of 491 Legionnaires' disease cases diagnosed in VHA facilities from 2014 to 2016, 67 percent were patients who had no VHA exposure, 3 percent had definite VHA exposure, and 31 percent had "possible" VA exposure (most of these cases were outpatient, making exposure difficult to attribute to a particular source). Most of the "definite" VHA exposure patients were in long-term care, which is a high-risk healthcare setting. According to Gamage et al. (2018), the total and the non-VHA associated Legionnaires' disease rates increased from 2014 to 2016. This was true both when calculating the number of cases either per total number of VHA enrollees or per number of VA enrollees who *used the system*. These cases were thought to be community-acquired Legionnaires' disease. In contrast, the rate of *VHA-associated* cases of Legionnaires' disease with overnight exposure decreased from 2014 to 2016. While it cannot be proven that implementation of the 1061 Directive was the cause for this decrease, it did occur over the time period of implementation of the Directive.

The environmental data collected from 2014 to 2016 are not yet published but were made available on a preliminary basis during a presentation to the Committee on October 22, 2018, and at a subsequent conference (Gamage and Roselle, 2018, 2019). Included are *Legionella*, biocide, temperature, and pH data collected quarterly from at least ten hot- and ten cold-water samples. In fiscal years 2015 to 2017, almost 150,000 samples were recorded from routine quarterly testing representing data from 790 buildings. Forty-four (44) percent of the 361 buildings with three full years of data had no detection of *Legionella*. In fiscal year 2015, *Legionella* was detected in less than 8 percent of the samples, and the percentage of positive *Legionella* samples decreased significantly over the three-year period (fiscal years 2015 to 2017).

These clinical and environmental data sets being collected by the VHA are unique in their number of sites and their broad scope. Their comprehensive analysis offers enormous potential for understanding the effectiveness of measures required by the Directive to control *Legionella* contamination of water systems and to monitor risk of Legionnaires' disease for individuals.

Centers for Medicare & Medicaid Services Memorandum

The Centers for Medicare & Medicaid Services (CMS) have regulatory authority over hospitals, critical access hospitals, and long-term care facilities that receive Medicare or Medicaid funds. The pertinent regulations include 42 CFR §482.42 for hospitals, which states: "The hospital must provide a sanitary environment to avoid sources and transmission of infections and communicable diseases. There must be an active program for the prevention, control, and investigation of infections and communicable diseases." Similarly, 42 CFR §483.80 for skilled nursing facilities and nursing facilities states: "The facility must establish and maintain an infection prevention and control program designed to provide a safe, sanitary, and comfortable environment and to help prevent the development and transmission of

communicable diseases and infections." And finally, 42 CFR §485.635(a)(3)(vi) for critical access hospitals states that policies must include: "a system for identifying, reporting, investigating, and controlling infections and communicable diseases of patients and personnel."

On June 2, 2017, CMS wrote a directive that requires Medicare-certified healthcare facilities to have water management policies and procedures to reduce the risk in building water systems of growth and spread of *Legionella* and other pathogens (e.g., *Pseudomonas*, *Acinetobacter*, *Burkholderia*, *Stenotrophomonas*, nontuberculous mycobacteria, fungi) (CMS, 2017). The directive endorsed the American Society of Heating, Refrigerating and Air-Conditioning Engineers' (ASHRAE) 188 standard and mentioned the CDC (2017) toolkit to aid hospitals and other facilities in implementing the ASHRAE standard. Quoting from the memo: "Healthcare facilities are expected to comply with CMS requirements to protect the health and safety of its patients. Those facilities unable to demonstrate measures to minimize the risk of Legionnaires' disease are at risk of citation for non-compliance with the CMS Conditions of Participation. Accrediting organizations will be surveying healthcare facilities deemed to participate in Medicare for compliance with the requirements listed in this memorandum, as well, and will cite non-compliance accordingly." In accordance with ASHRAE 188, the CMS memo does not explicitly require hospitals and nursing homes to conduct monitoring of *Legionella* within facilities.

In the United States there are more than 15,000 nursing homes and 4,784 hospitals registered with Medicare.[2] These numbers do not represent individual buildings because one hospital can have multiple buildings associated with it. These numbers, however, are similar to those cited in Circle of Blue (2018), which says that the CMS memo applies to 15,688 nursing homes and 6,862 hospitals, which includes children's care, psychiatric, and rehabilitation centers. According to the Circle of Blue report from December 9, 2018, it is too early to know whether hospitals are complying with the CMS memo. Many of the larger hospitals that receive Medicare and Medicaid reimbursements independently instituted *Legionella* management in their buildings years ago. Given that nursing homes are surveyed for compliance annually and hospitals are surveyed only every three years, it may take time for CMS to obtain enough data to establish the level of compliance. CMS has yet to announce how many of the compliance surveys completed since the memo's publication in 2017 found inadequate *Legionella* management plans.

Given its brevity and reliance on ASHRAE 188, the CMS memo does not provide any specifics; for example, it does not indicate the required temperatures for building hot-water systems (unlike the VHA Directive 1061, which is very prescriptive, as detailed above). However, CMS defines "immediate jeopardy" with respect to scalding as "access to hot water of sufficient temperature to cause tissue injury" (CMS, 2014). This vague definition has been interpreted differently by states, from South Dakota allowing an operating hot-water temperature of 125°F (52°C) to other states that have defined immediate jeopardy as 105°F (40.5°C). CMS could have a rapid, profound impact on *Legionella* management in facilities that receive Medicare and Medicaid reimbursements if it specified that immediate jeopardy is not reached until hot-water temperatures are greater than 125°F (52°C) (see Table 4-3).

New York City and New York State Cooling Tower Regulations

The first regulation in the United States to require registering and monitoring cooling towers for *Legionella* was enacted in 2005 in Garland, Texas, for cooling towers associated with multifamily housing; it was later revised to include hotels and places of accommodation (Whitney et al., 2017). The ordinance was simple and implemented at low cost to the health department, resulting in a decrease in the number of contaminated cooling towers over time. All testing was required to be independent of those responsible for maintenance.

[2] See data.medicare.gov.

Ten years later, New York City passed similar legislation following recent outbreaks of Legionnaires' disease caused by cooling towers in the city. Local Law 77 when into effect in 2015, with the New York City Department of Health and Mental Hygiene implementing the law. New York State followed by creating Title 10 Part 4 of the New York Codes of Rules and Regulations (10 NYCRR Part 4 "Protection against *Legionella*"). Final adoption of the New York State regulations occurred in July 2016. The state's regulations apply statewide, including in New York City, such that there is overlap between the city and state regulations.

The New York City and New York State regulations require cooling tower owners to take the following actions:

1. Register existing and new cooling towers with the city and state.
2. Sample each cooling tower for *Legionella* every 90 days.[3] Notify the city within 24 hours if *Legionella* culture results are greater than 10^6 CFU/L; the state every 90 days while the cooling tower is in operation; and the local health department within 24 hours for any result greater than 10^6 CFU/L.
3. Perform daily chemical treatment of system water.
4. Monitor temperature, pH, conductivity, and biocides at least three times per week. Microbial monitoring (heterotrophic plate counts) must be performed weekly, wetted surfaces are visually inspected weekly, and chemical treatment equipment is also checked.
5. Inspect the cooling towers every 90 days and obtain annual certification, by a qualified person.
6. Develop and follow maintenance program and plan in line with the ASHRAE 188 standard.
7. There are various other requirements for drift eliminators, materials, cleaning, and documentation.
8. If an owner does not register, have a maintenance program and plan, obtain certification, disinfect, perform or obtain culture sampling and analysis, or inspect a cooling tower within the required time and manner, New York State or the local health department may determine that the situation constitutes a nuisance and may take action, as authorized by law. New York State or the local health department may also take any other action authorized by law, including imposing any and all applicable civil and criminal penalties.

More details on reporting, enforcement, penalties, and updates can be found in the official documentation (NYC, 2016a,b).

As of April 2019, there were approximately 6,100 cooling towers registered in New York City and about 11,000 in New York State (including New York City). Currently, results from the New York City *Legionella* sampling are not publicly available, but this will change in October 2019. Cooling tower data for New York State are publicly available.[4] Both the city and state programs have seen progress in implementation and adherence to the existing regulations. For New York State, rates of cooling tower compliance with current regulations increased from 30 percent in 2017 to 67 percent in 2018. In New York City, the cooling tower registry has been invaluable in providing real-time information for Legionnaires' disease cluster response. Promptly locating cooling towers and having a history of operations and

[3] All monitoring thresholds in this chapter are expressed in CFU/L for consistency. However, this is not meant to imply that all samples are 1 L in volume. Sampling protocols differ between jurisdictions.

[4] See https://health.data.ny.gov/Health/Registered-Cooling-Towers-Beginning-August-2015/24a4-muw7.

maintenance records has provided valuable information for identifying potential sources and evaluating risk. Additional benefits of the regulations include:

- An increase in the proportion of cooling tower systems that have a maintenance program and plan;
- An increase in the proportion of cooling tower systems that have water treatment including biocide, anti-corrosion, and anti-scaling treatment;
- An increase in the proportion of cooling tower systems that document and maintain operational records; and
- An increase in *Legionella* and bacterial monitoring during cooling tower operation.

The proportion of cooling towers testing positive for *Legionella* in samples collected by the department is typically below 30 percent and has remained relatively stable in the first two years of implementation of the regulations.

New York State Healthcare Facility Requirements for *Legionella*

As of July 2016, all general hospitals and residential healthcare facilities in New York State are required to perform an environmental assessment, prepare and implement a sampling and management plan to sample their potable water systems for *Legionella*, and institute control measures in the event of a *Legionella* exceedance. The New York State regulations apply to buildings of general hospitals that provide in-patient services or buildings of residential healthcare facilities that provide a "health-related service," such as lodging, board, and physical care. The regulation does not apply to administrative buildings of such facilities, general hospital buildings that only provide outpatient services, or to diagnostic and treatment centers providing only outpatient services.

All covered facilities must perform an environmental assessment of the facility using a specified environmental assessment form prior to providing services, and when specified by the New York State Department of Health (NYS DOH), such as when there are suspected or actual cases of legionellosis, after certain construction and repairs, and after expansion and relocation of certain medical units. Furthermore, all covered facilities must adopt and implement a *Legionella* culture-based sampling and management plan for their potable water systems. New covered facilities must adopt this plan prior to providing services. *Legionella* culture sampling and analysis must occur at 90-day intervals during the first year of sampling and management plan implementation, and annually thereafter. Portions of any potable water system that serve hematopoietic stem cell transplant or solid organ transplant patients must continue to be sampled and analyzed at intervals not to exceed 90 days. *Legionella* culture sampling and analysis must also occur in a timeframe determined by the state health department.

All *Legionella* culture analyses must be performed by a laboratory that is approved to perform such analysis by the New York State Environmental Laboratory Approval Program (ELAP). When 30 percent or more of *Legionella* culture samples contain *Legionella,* regardless of species, facilities are required to institute control measures, resample their water system, and notify the NYS DOH. (This 30 percent threshold stems from the Allegheny County guidance mentioned in a subsequent section.) The covered facility must maintain the required environmental assessment and any associated sampling results on the facility premises for at least three years. These records must be made available to the NYS DOH immediately on request. The department may conduct an assessment and/or *Legionella* culture sampling and analysis of the potable water system at any time. A violation of any provision of the regulation is subject to civil and criminal penalties. Each day that an owner remains in violation of any provision of this Subpart

constitutes a separate and distinct violation of each such provision.[5] Citations during routine facility inspections are issued by healthcare facility surveyors, who are tasked with implementing the 2017 CMS memo on *Legionella* prevention in healthcare facilities. NYS DOH reports that letters of non-compliance sent to healthcare facilities have had positive effects (mostly immediate responses and compliance).

Legionella data stemming from this regulation are not currently available on a public platform, nor is direct reporting of culture data to the NYS DOH a requirement of the regulations. NYS DOH is developing measures to evaluate the effectiveness of the regulations, while recognizing that several more years of data will be needed to identify meaningful trends.

Plumbing Codes

Plumbing codes dictate almost every facet of building plumbing design and installation, including insulation, materials used, allowable pipe size and length, allowable volume from a hot-water source to a tap, control of heated water systems including storage and circulation, drain water heat recovery, and commissioning. Building owners have to be in compliance with plumbing codes when a building is being built or renovated; hence, codes are mainly enforceable prior to a certificate of occupancy being signed. Some plumbing codes can be used to partially manage *Legionella* in building water systems, and those codes are the focus of this section. Plumbing codes can provide a backstop for buildings, such as residences, that would otherwise not fall under any guidance documents or other enforceable policy for *Legionella* management.

The three main plumbing codes are the International Plumbing Code (ICC, 2017), the Uniform Plumbing Code (IAPMO, 2018a), and the National Standard Plumbing Code (IAPMO, 2018b). Plumbing codes are adopted at the state level but are generally enforced at the county or municipal level by the relevant inspection entity. The International Plumbing Code is revised every three years by the International Code Council (ICC). It has been adopted at the state or local level in 35 states. The Uniform Plumbing Code was developed by the International Association of Plumbing and Mechanical Officials (IAPMO), also on a three-year cycle, and has been adopted in 13 states, mainly in the west and northwest United States including California. The National Standard Plumbing Code was developed by the Plumbing Heating and Cooling Contractors of North America (which was recently purchased by IAPMO) and has been adopted in New Jersey and parts of Maryland and is not discussed further here. It should be noted that in states with state-wide codes, these codes may be modified by local ordinances. For example, California adopts a statewide plumbing code, but in Colorado each jurisdiction (individual cities and counties) can adopt different codes. For all three codes, product manufacturers tend to drive the code change proposals.

Historically, plumbing codes were not written with the goal of managing building water systems for *Legionella*. In fact, some codes (unintentionally) work against the control of *Legionella*. For example, pipe-sizing requirements that were set more than 50 years ago remain unchanged, even though plumbing fixture flow rates, flush volumes, and appliance volumes have been reduced every decade since the 1950s. Because there have been significant reductions in average residential water use since the 1980s without concomitant reductions in pipe sizes, household flow rates have been drastically reduced. There are many unintended consequences of having lowered water use in conjunction with oversized pipes. Foremost among them is that hot water takes much longer to arrive at taps. As discussed in Chapter 4, increased water residence times in premise plumbing systems can lead to conditions highly conducive to *Legionella* growth. A related issue is that plumbing codes were created under the assumption of fixed-orifice de-

[5] See https://www.health.ny.gov/environmental/water/drinking/ legionella/hospitals_health_care.htm, https://regs.health.ny.gov/content/subpart-4-2-health-care-facilities.

vices. Today, however, many devices have pressure-compensating aerators, which provide a constant flow rate to the consumer above 20 psi pressure. The use of such aerators should allow builders to more correctly size pipes, but these corrections have not yet found their way into plumbing codes.

A second plumbing code feature that affects *Legionella* management is the temperature requirement, particularly in public bathrooms. Since 2006, the International Plumbing Code has stipulated that, for public bathrooms, the temperature of hot water at handwashing stations must be between 85°F (29.4°C) and 110°F (43.3°C)—the definition of tempered water. Hence, the codes require delivery of water in the temperature range optimal for *Legionella* growth. The 2018 International Plumbing Code defines "hot water" as greater than 110°F (43.3°C), while the Uniform Plumbing Code defines it as 120°F (48.9°C).

To meet the temperature requirements, plumbing codes mandate the use of certain devices, such as thermal mixing valves, to deliver water of various temperatures to various locations in premise plumbing, particularly near end-use points and at emergency stations. The International Plumbing Code also requires thermal mixing valves for every public handwashing sink. As discussed in Chapter 4, these devices tend to fail without warning in a few years, especially when substandard devices are used. The plumbing codes do not require periodic testing of thermal mixing valves. Another device requirement found in plumbing codes is for combination tub and shower valves that deliver cold, warm, or hot water. Often, the highest flow rate than can be achieved with these devices is for the mixed temperature water, not the hot water, leading to oversized pipes.

Material requirements in plumbing codes can affect *Legionella* management. Both the International Plumbing Code and the Uniform Plumbing Code dictate materials to be used for piping, devices, and joints, among others, with the list of acceptable materials being determined mainly by the manufacturers. To avoid future liability, manufacturers tend to avoid promoting materials that leach compounds. Nevertheless, it would be preferable if the requirements in the plumbing codes were based on robust studies of what materials are more or less favorable to growth of *Legionella*.

Indeed, to have the greatest positive effect on *Legionella* management, plumbing code changes would suggest new ways to design plumbing systems before a building is constructed. The main tenet of an ideal code would be to correctly size pipes to reflect lowered water use and measured pressure drops in systems with modern pipe materials and fittings. In addition, builders should be incentivized, perhaps with energy credits, to build compact plumbing cores with no more than ten feet between the hot-water source and fixtures. Another practice to incentivize would be the use of electric tankless water heaters that would require no mixing valves, or the use of heat trace to maintain adequately high temperatures in pipe branches. Finally, the plumbing codes could define tempered water to be between 120°F and 130°F (43.3°C to 48.9°C), such that water delivered to taps would not be in the range of optimum *Legionella* growth.

GUIDANCE DOCUMENTS

Many guidance documents that outline steps to manage *Legionella* in building water systems have appeared over the past five years. The most prominent of these are discussed below, including the ASHRAE 188 standard, guidance from the American Industrial Hygiene Association (AIHA, 2015), National Sanitation Foundation International (NSFI) standard 453, and the guidelines from the Allegheny County Health Department in Pennsylvania.

In general, like the VHA Directive, the major guidance documents take a risk-based approach to managing *Legionella* in building water systems. To a greater or lesser extent, they each follow the

general principles of risk management and require development of a plan or program to mitigate the risk (variously called water safety plans, water management programs, and other terms). Each plan or program typically follows the basic steps shown below (which are similar to those within the VHA Directive):

- Establish a program team.
- Describe each water system.
- Analyze where potential hazards may exist, develop, or propagate.
- Identify control measures and where they should be applied to stay within limits.
- Monitor certain parameters (not necessarily *Legionella*) to determine if control measures are working.
- Confirm that the program is being implemented as designed (verification) and that the program effectively controls the hazardous conditions (validation).
- Document everything.

Depending on the guidance document and its developers, certain specifics for managing *Legionella* in water systems are enhanced or expanded to meet the needs of their organizations. Interestingly, each guidance document is sufficiently vague to permit individual users the flexibility and latitude to address those program aspects either unique or specific to their own buildings or uses. On the other hand, the lack of strong commonality among these documents on the details and specifics of managing *Legionella* in water systems collectively creates confusion. In fact, one of the primary drivers for the creation of the CDC's tool kit (CDC, 2017) was to specify how to actually apply the ASHRAE 188 standard. Highlights of each major guidance document and their particular differences are identified below.

Allegheny County Health Department *Legionella* Guidelines

Guidance documents produced by the Allegheny County Health Department (1993, 1997, 2014) were some of the first to address *Legionella* in building water systems. These guidance documents say that all facilities should take a risk management approach regarding *Legionella* in their water systems. A key recommendation is development of a water safety plan, the elements of which are described above. However, there is no requirement in the water safety plan for a program team or for documentation in the Allegheny County Health Department guidance. The guidance specifically outlines different types of control measures, from thermal disinfection to point-of-use filters. Finally, unlike the other guidance documents, this guidance includes a section on managing Legionnaires' disease in patients.

The Allegheny County Health Department guidance documents are the basis for the 30 percent positivity rule that has permeated many other guidance documents as well as the previously described New York State regulations for healthcare facilities. Best et al. (1983) found that whenever monthly site positivity of environmental testing for *Legionella* in a large building exceeded 30 percent, cases of Legionnaires' disease appeared in those months. Furthermore, when positivity fell to 20 percent or less, no cases of disease were observed. The 30 percent value is very controversial, as is noted in the 2014 guidance from Allegheny County. The Committee notes substantial difficulties with using this guidance, including the fact that the analysis has not been repeated and validated elsewhere and it has frequently been applied in situations that lack an adequate number of samples. While it was useful in the 1990s, comprehensive guidance based on *Legionella* concentration along with the frequency of detection would be more consistent with the available science.

ASHRAE Standard 188 on *Legionella* in Building Water Systems

The ASHRAE 188 standard (ASHRAE, 2015) is a guidance document for all types of buildings and their water systems, with the exception of residential single-family homes. The core of the standard is for each building to have a water management program that has seven elements, as described in the bulleted list above. For ASHRAE 188, the most important element is the creation of a program team to make decisions about other aspects of the water management program. Notably, ASHRAE 188 does not specify that monitoring of *Legionella* in the building water system is necessary unless the program team decides that it is. Instead, to manage *Legionella* occurrence, the focus is on controlling and monitoring certain physicochemical factors (e.g., temperature, disinfectant residual, and maintenance).

ASHRAE 188 considers individual types of water systems, from the potable water system to cooling towers and evaporative condensers, spas, fountains, and aerosol-generating misters. There are some peculiarities for each type of water system, and the standard states that details are given in ASHRAE Guideline 12 (ASHRAE, 2000). The standard also covers requirements for *designing* building water systems, such as documenting potential hazards in all major water systems, as well as documentation of all the water systems themselves upon installation, including what was built and where, what materials were used, and corresponding manuals. Detailed instructions for commissioning, including flushing and disinfection, have to be provided.

Annexes treat specific issues or building types, such as Annex A for healthcare facilities. This Annex calls for a yearly evaluation of the likelihood of legionellosis in healthcare facilities, which is not mentioned in the main part of the standard. Annex C states that any *Legionella* testing must be done by accredited laboratories.

CDC Tool Kit

The CDC tool kit (CDC, 2017) was created to help building owners and managers develop and implement a water management program to reduce a building's risk for growing and spreading *Legionella*. The toolkit both simplifies and explains ASHRAE 188, and it applies the principles to healthcare facilities. For example, there are a series of questions to be answered to determine if a particular building is at risk of *Legionella* contamination, as well as sections that specify how to assemble the program team. There is a useful flow diagram of a building's water system, which is then used repeatedly throughout the water management program to identify areas that may be susceptible to *Legionella* growth and to show where control measures will be applied. Examples are given of when a control limit is exceeded and corrective actions are necessary, and what to do if *Legionella* is found in a building. The toolkit provides further details for healthcare facilities, which are more likely to suffer adverse effects from *Legionella* contamination.

AIHA Guidance on *Legionella* in Building Water Systems

AIHA (2015) is a guidance document intended to help building managers anticipate, recognize, evaluate, and control *Legionella* in buildings. It covers premise plumbing; cooling towers and evaporative condensers; hot tubs, whirlpools, and spas; decorative fountains and water features; humidifiers; the water supply system; and sprinklers, eyewash stations, and safety showers. The guidance differentiates its approach to *Legionella* management (which comes from the industrial hygiene field) from what is labeled

"current health practice" that goes into action only after a case of Legionnaires' disease has occurred. The guidance describes such "current health practice" as appropriate for diseases transmitted between persons, but ineffective when the environment is the source of the etiologic agent. Hence, AIHA (2015) focuses on identifying sites of *Legionella* amplification and exposure pathways using measurements of viable *Legionella* bacteria; it does not endorse sampling surrogates such as disinfectant residual.

Similar to other guidance documents, AIHA (2015) is based on risk assessment, but it tends to be more descriptive. It specifically calls for *Legionella* samples to be collected (to be assayed via the culture method) from selected water systems on an ongoing basis to determine the effectiveness of control strategies and identify potential hazards. AIHA (2015) recommends that, although PCR techniques can be used as a complementary analysis, they should not replace culture-based methods. Table 5-1, taken from the guidance document, provides levels of *Legionella* that can be thought of as action levels to compare to routine sampling results. For all water system types (except cooling towers), a measured concentration below 1 CFU/mL (1,000 CFU/L) is considered to be at the detection limit. Between 1 and 10 CFU/mL (1,000 to 10,000 CFU/L), *Legionella* amplification could be possible. A sample above 10 CFU/mL (10,000 CFU/L) indicates that amplification has occurred, and action needs to be taken (for cooling towers, the document suggests that 100 to 1,000 CFU/mL [10^5 to 10^6 CFU/L] is indicative of possible amplification). These values mirror those of the Occupational Safety and Health Administration (OSHA), which used to suggest guidelines for *Legionella* to assess the effectiveness for water system maintenance but no longer does.[6]

TABLE 5-1 AIHA Data Interpretation Guidelines

Sample Source	Non Detectable	Acceptably Low*	Action	Possible Amplification	Action	Indicates Amplification	Action
Humidifiers & Misters	<1 CFU/mL	<1 CFU/mL	1	1–10 CFU/mL	2	>10 CFU/mL	3
Decorative Fountains and Water Features	<1 CFU/mL	<1 CFU/mL	1	1–10 CFU/mL	2	>10 CFU/mL	3
Hot Tubs, Whirlpools and Spas	<1 CFU/mL	<1 CFU/mL	1	1–10 CFU/mL	2	>10 CFU/mL	3
Potable Water	<1 CFU/mL	<10 CFU/mL	1	10–100 CFU/mL	2	>100 CFU/mL	3
Industrial Working Fluids	<1 CFU/mL	<10 CFU/mL	1	10–100 CFU/mL	2	>100 CFU/mL	3
Cooling Towers & Evaporative Condensers	<10 CFU/mL	<100 CFU/mL	1A	100–1,000 CFU/mL	2A	>1,000 CFU/mL	3A

*May be limited by *Legionella* levels in the building source water supply (e.g., municipal water). Action levels (see AIHA 2015 for complete details): (1) Continue to monitor as per the plan. (2) If no cases of legionellosis, reassess maintenance and treatment plans; make adjustments as necessary. If cases of legionellosis occur, take immediate steps to clean and disinfect the system. Notify appropriate health authorities. (3) Take immediate steps to clean and disinfect the system. Adjust control plan as needed. SOURCE: AIHA (2015).

Unlike the other guidance documents, AIHA (2015) discusses side effects of various treatment techniques that need to be taken into account. The guidance also provides considerable detail about how to protect workers and building occupants (such as with point-of-use devices) during remediation activities.

[6] See https://www.osha.gov/dts/osta/otm/otm_iii/otm_iii_7.html#app_iii:7_3.

NSFI Standard 453 for Cooling Towers

NSFI standard 453 (NSFI, 2017) provides minimum practices for treating, operating, and maintaining cooling towers to avoid *Legionella* growth. The standard uses the terms *program* (all the conducted activities) and *plan* (the documentation of the program) and adheres to the bulleted list of risk management elements discussed previously.

Standard 453 requires treatment of cooling towers with an oxidizing biocide as well as maintenance of pH, corrosion control, scale and deposit control, and conductivity. Startup procedures for cooling towers are outlined, including initial startup and after a system shut-down. Routine inspection, service, and maintenance are outlined, including weekly, quarterly, and when there is an issue. Cycles of concentration are a key operating parameter for cooling towers that need to be monitored, managed, and documented.

Monitoring of biocides is required as part of standard 453, as is testing for heterotrophic plate counts and *Legionella*. If concentrations of *Legionella* are less than 10 CFU/mL (10,000 CFU/L), no action is needed. If concentrations are between 10 and 100 CFU/mL (10,000-100,000 CFU/L), then the entire program needs to be reviewed and on-line remedial treatment is needed. Between 100 and 1,000 CFU/mL (10^5 to 10^6 CFU/L), a visual inspection is also required (in addition to the above) to determine if full draining and repair are required. Greater than 1,000 CFU/mL (10^6 CFU/L) requires off-line remedial treatment, and the standard describes the conditions for completely shutting down the cooling tower. This standard will be superseded by NSFI standard 444, which, when released, will cover all building water systems, not just cooling towers.

REGULATIONS AND POLICIES FROM OTHER COUNTRIES

Although there are no nationwide regulations for *Legionella* management in the United States, several other countries, notably those in Europe, have enacted regulations meant to manage *Legionella* in various types of water systems. Some of these mirror the document produced by the World Health Organization (WHO) in 2007, which advocated that buildings have a water safety plan and monitor temperature, pH, and (for validation purposes only) *Legionella*. This section summarizes information gathered by the Committee from representatives of six countries about their *Legionella* laws, regulations, guidance documents, and codes. Their presentations described the regulations, noted what water systems they apply to, and discussed the extent of compliance with the regulations. Each presenter described the environmental monitoring that must accompany the regulations, including threshold levels above which remedial or preventive action is taken. Finally, they talked about whether the regulations have had an impact on reducing rates of Legionnaires' disease or environmental concentrations of *Legionella*. The six countries are Australia, Canada, France, Germany, the Netherlands, and the United Kingdom.

The European Working Group for *Legionella* Infections (EWGLI) published guidelines for the prevention, control, and investigation of infections caused by *Legionella* spp. (EWGLI, 2017), but their recommendations have no legal standing. A new European Drinking Water Directive was developed in 2018 (EU, 2018), which makes risk assessment in domestic building water systems obligatory. This risk assessment includes risks linked to products and materials in contact with drinking water and monitoring for lead and *Legionella*. *Legionella* was included in the directive because (1) it has been found by the WHO to cause the highest health burden of all waterborne pathogens in the European Union (EU) and (2) the European Centre for Disease Prevention and Control recommended regular checks and appropriate control measures to human-made water systems to prevent cases of Legionnaires' disease at

tourist accommodation sites, hospitals, long-term healthcare facilities, or other settings where sizeable populations at higher risk may be exposed (ECDC, 2017). How *Legionella* sampling and analysis should be performed is also defined in the new EU drinking water directive. One-liter samples must be taken at the consumer's tap without prior flushing and analyzed for culturable *Legionella* according to ISO 11731 (ISO, 2017). When the culturable *Legionella* number in a drinking water sample does not comply with 1,000 CFU/L, resampling has to be performed, and new samples have to be screened for *L. pneumophila*. When *L. pneumophila* is detected, action has to be taken. If *L. pneumophila* is not detected during resampling and cultivable *Legionella* is less than 10,000 CFU/L, no action has to be taken. Each EU member state determines what action their country will be take when these action levels are exceeded. After the first vote in the EU parliament, several amendments were suggested, but the expectation is that the new directive will be adopted soon by the EU parliament. EU countries without *Legionella* regulations will have to comply with the new action levels. Those countries that already have *Legionella* regulations in place (discussed below) tend to be more strict than the new European directive; as a result, they will not have to modify their regulations.

The Netherlands

Four laws in the Netherlands were revised to include *Legionella* spp., in each case following an outbreak. For the first *Legionella* outbreak, in 1999, a hot tub on display in Bovenkarspel was found to be the source of the bacteria, resulting in more than 200 cases of Legionnaires' disease with 32 fatalities (den Boer et al., 2002). This outbreak led to the addition of regulations to the Drinking Water Act, the Hygiene and Safety Act, and the Safety at Work Act. In 2006, a second outbreak caused by a contaminated cooling tower in Amsterdam led to the creation of new regulations in the Environmental Protection Act. *Legionella* is the only pathogen in premise plumbing to be a target of Dutch regulations.

The Drinking Water Act regulations apply only to buildings labeled "priority premises" such as hospitals and other healthcare facilities with overnight stays, hotels and other accommodations that house more than five people, truck stops with showers, harbors and marinas with showers, asylum centers, and prisons. The Act requires that a risk management plan for the building be created, and include such risks as water stagnation, tepid temperature, formation of aerosols, presence of high-risk individuals, past cases of Legionnaires' disease, whether the building accommodates travelers, whether it is used for temporary events, and lack of proper maintenance. The owner of the building must periodically update the risk management plan. Management plans are generally created by certified consultants and must conform to technical guidelines (e.g., about how, when, and where to sample).

The highest priority control methods discussed in the Act are temperature control, flushing, UV treatment, and filtration. Secondary methods include electrochemical treatment such as copper-silver ionization. Chlorination is a tertiary treatment, although it conflicts with the long-standing Dutch paradigm of not carrying a disinfectant residual in finished water. In terms of temperature control, cold water must be maintained at less than 25°C, while hot water must be higher than 60°C throughout the hot-water system. The Act requires that buildings be sampled every six months for *Legionella* spp. using culture methods. If the concentrations are less than 100 CFU/L, no further action is taken. Above 100 CFU/L and especially above 1,000 CFU/L, certain response actions are required such as informing the users of the building, taking appropriate measures to prevent a public health threat, and notifying the Inspectorate. It is noteworthy that these numbers were chosen for practical reasons and are not health-based. The detection limit for the laboratories is 100 CFU/L, and 1,000 CFU/L was a practical number larger than 100 CFU/L.

The Hygiene and Safety Act is similar to the Drinking Water Act, but it applies to swimming and bathing facilities above a certain size and where aerosols are dispersed. It does not apply to hot tubs. As above, a risk management plan is required, and the monitoring requirements are the same as in the Drinking Water Act. Major differences are that certification of consultants is not required, there are no technical guidelines available, and any disinfection method can be used.

The Environmental Protection Act applies to wet cooling towers only. Like the previous two Acts, it requires a risk assessment and management plan that includes treatment and monitoring. Technical guidelines are also available to help guide the creation of such plans. However, no specific treatment is required as long as an effective method is used. Similarly, the monitoring requirements are loose, with no specific threshold above which action must be taken and no recommended frequency of monitoring. This Act does not require that the consultant creating the risk management plan be certified, but the plan must be updated when cooling tower operations change or the surroundings change. The Act requires all cooling towers built after 2010 to be registered; currently, about one-third of all towers are registered.

The Safety at Work Act aims to protect workers from *Legionella* exposure. As with the other Acts, a risk assessment and management plan is needed. The Act does not require particular treatment methods or monitoring frequency, although it does specify a threshold of 100 CFU/L as measured by culture.

Implementation of the Drinking Water Act regulations has been tracked since 2009 via facilities inspections. The trends suggest that more and more of the facilities are becoming "no risk." For cooling towers, data from 2011 and 2015 suggest that municipalities are identifying their cooling towers (88 percent) and that a substantial portion of towers are being registered (30 percent). On the other hand, inspections lag behind, with two-thirds of inspectorates not visually inspecting their cooling towers. Less information is available on implementation of the Hygiene and Safety Act and the Safety at Work Act. All of the regulations, except the Safety at Work Act, are supposed to be enforced via inspections, which could then lead to warnings, fines, and facility closure.

There is no information on whether these regulations have affected either rates of Legionnaires' disease or environmental sampling of *Legionella*. In the Netherlands in general, Legionnaires' disease rates are still going up (they were 3.3/100,000 in 2017—ECDC, 2019), although travel-associated cases seem to have leveled off. Many believe the national data are insufficient to evaluate the impact of the regulations, particularly given the lack of data on both Legionnaires' disease rates and *Legionella* concentrations collected at the same location.

Germany

German regulations for *Legionella* spp. extend drinking water regulations into premise plumbing. In 1987, the German Federal Health Department said that drastic reduction in *Legionella* concentrations was necessary to reduce infections, further emphasizing that the goal should be "as low as reasonably achievable." The country's federal Protection Against Infection Act regulates water for consumption as well as swimming pools and bathing water and all wastewater treatment plants. For drinking water in particular, there is not only the Protection Against Infection Act, but also the EU Drinking Water Directive (discussed previously), a German ordinance on the quality of water for human consumption, and proposals of the German federal Environmental Protection Agency. The Protection Against Infection Act requires a *Legionella* risk management plan for every large building that has more than 400 liters of hot water or more than three liters of hot water between the water heater and the last tap at the end of the pipe. Healthcare facilities are required to maintain a temperature of 60°C at hot-water heaters and 50°C at distal points of hot-water systems. To facilitate compliance with the regulations, there are at least six technical standards that stem from the Act.

The federal German Emissions Control Act and technical guidance cover every open cooling tower. They have similar requirements for a management plan, monitoring, and concentration thresholds above which action must be taken.

The Protection Against Infection Act also targets *Legionella* that may stem from the disposal of wastewater, both industrial and municipal. The impetus for its implementation was a 2013 outbreak caused by outflow from a wastewater treatment plant. Specifically, a brewery was disposing of warm (38°C) wastewater, which was found to have high levels of *Legionella* (10^7 CFU/L), and the receiving river was contaminated for 12 kilometers downstream of the outfall with some 200,000 CFU/L of *Legionella* (Maisa et al., 2015). Therefore, it was decreed that wastewater treatment plants must be monitored for *Legionella* by culture methods. It has now become evident that warm wastewaters from breweries, paper mills, and sugar processing plants can have very high *Legionella* concentrations.

These regulations require monitoring of environmental samples for *Legionella* spp. at various locations in a building; measured concentrations must be less than 100 CFU/100mL (1,000 CFU/L). This concentration is called a technical action level and, as in the Netherlands, it is not a health-based number, but rather a value above which certain additional precautions must be taken, including checking the sanitary and technical condition of the drinking water installation in the form of a risk analysis and informing public health agencies.

The regulations are accompanied by many technical guidance documents and rules for planning and construction, much like plumbing and building codes in the United States. Code of Practice W551 (DVGW, 2004) addresses measures to reduce *Legionella* growth in buildings and is particularly relevant, as it recommends that one keep the volume of stored hot water small, keep the hot water hot and the cold water cold, avoid stagnation, maintain and inspect, rehabilitate, and perform more microbiological examinations. There is also a code for remedying microbial irregularities in drinking water installations (W556, 2015), and one for cleaning and disinfecting drinking water installations (W557, 2012). Guideline 6023 deals with qualifications and staff training. Cooling towers are subject to a Cooling Tower Code of Practice, which has threshold concentrations above which remedial actions are required.

All of Germany has to comply with the regulations, and compliance is high because building owners can be punished by law for not complying. Implementation of the regulations in large cooling towers and most building water systems is now broad, although the implementation is not as widespread for small cooling towers. The sewage regulations are only being enforced in North Rhine Westphalia. In general, the government tries to educate water consumers about *Legionella* by posting information on every public health department's website. If monitoring data are above the action level for a large apartment building, the building owner has to inform the occupants. As a result, people have become much more aware of *Legionella*. There are also guidelines for the homes of immunosuppressed people.

Germany has been trying to determine the impact of their regulations with the LeTriWa (Legionellen in der Trinkwasser) Project conducted in Berlin from 2017 to 2019, which is reviewing outbreak reports and follow up surveillance. There is no indication that cases of Legionnaires' disease have gone down since the regulations came into effect. In 2017, the case rate in Germany was 1.6 per 100,000 people (ECDC, 2019). However, the LeTriWa study has shown that since the regulations went into effect, the percentage of buildings that have culturable *Legionella* has declined from 70 percent to just 10 percent (Exner, 2018). There is also anecdotal evidence that a hospital that had 11 cases of Legionnaires' disease in 1990 has had no cases since the implementation of controls (Exner, 2018).

England

England's regulations for *Legionella* management have evolved from two pages of guidance in 1980 to hundreds of pages today. The primary impetus for creating regulations was the 1985 outbreak of Legionnaires' disease at Stafford Hospital. Engineering guidance was then developed for cooling towers and evaporative condensers. In 1991, the Health and Safety Commission published the Approved Code and Practice for the Prevention of Legionellosis. In 1998, there was specific guidance for hot- and cold-water systems. In 2000, everything was combined into one guidance called L8, in which there are various levels of documents: laws, regulations, approved codes of practice, and technical guidance.

Much of the guidance stems from the 1974 Health and Safety at Work Act, which aims "to ensure the health and safety of employees and non-employees so far as reasonably practicable." Although the term "reasonably practicable" is ambiguous, it has held up over the years because the risks of Legionnaires' disease are significant and the consequences may be deadly, so high cost is justified. The various UK laws and regulations have many facets in common. The Control of Substances Hazardous to Health regulations have a series of requirements, including a risk assessment and control measures. The Management of Health and Safety at Work regulations also require risk assessment, more planning control, monitoring, and review of preventive measures. The Notification of Cooling Towers and Evaporative Condensers Regulations of 1992 are the only regulations that specifically address *Legionella.* Cooling towers must also be registered, and more than 90 percent are.

The main L8 regulations have four accompanying guidance documents that cover evaporative cooling systems, hot- and cold-water systems, spas and pools, and other systems. The regulations apply to all systems containing water likely to exceed 20°C for which there is a means of creating and transmitting water droplets that may be inhaled. The building types covered include shops, offices, factories, hospitals, industrial plants, entertainment facilities, and rented properties, among others, but private residences are excluded. Healthcare facilities have a slightly different set of regulations stemming from the Health and Social Care Act of 2008, a new set of regulations, a new Code of Practice, and new technical memos. To maintain their registration, all healthcare facilities must follow the Code of Practice. The latest version of the technical memo uses the terms water safety groups or water safety plans and highlights the role of climate change. It applies not just to *Legionella* but also to *Pseudomonas aeruginosa* and mycobacteria, among other pathogens.

The maintenance of an adequate temperature control regimen is presented as the preferred approach for *Legionella* control (Department of Health and Estates and Facilities Division, 2006a,b; HSE, 2013). Hot-water temperature leaving the water heater should be maintained at more than 60°C, temperatures in the return loop should be above 50°C, and temperature at determined sentinel points (including the farthest draw-off point) should exceed 50°C for all types of buildings and, for healthcare facilities, above 55°C after draw-off of one minute (HSE, 2013).

The monitoring requirements for cooling towers are quarterly sampling for *Legionella* spp. with a target of less than 100 CFU/L. Heterotrophic plate counts have to be less than 10,000/mL. For hot- and cold-water systems, *Legionella* tests are not required, although it is recommended if biocide failure has occurred, if there are high-risk individuals in the building, where biocides are used as the primary control and not high temperature, or if a case of Legionnaires' disease has been associated with the premises. At concentrations above 100 CFU/L and 1,000 CFU/L, certain actions have to be taken. More stringent requirements for monitoring of healthcare facilities and for spas and pools exist as well.

Health and safety executive inspectors enforce the Health and Safety at Work Act for hospitals, heavy industry, and manufacturing premises. Unfortunately, due to a shortage of funding, no preventive inspections are conducted. England has not had the resources to determine what percentage of buildings

are colonized with *Legionella*, although there is no reason to believe that the proportion of buildings with *Legionella* has changed. To make the regulations more enforceable, fines have been raised and are now in the millions of pounds. England has not measured the effectiveness of its *Legionella* regulations. In 2017, the case rate for Legionnaires' disease in the United Kingdom was 0.8 per 100,000 people (ECDC, 2019).

In England, specific *Legionella* regulations exist for dentistry, managed by the Care Quality Commission (CQC). To comply with these requirements of the CQC, the Department of Health's Health Technical Memorandum 05-01 (Department of Health and Estates and Facilities Division, 2013) requires all dental practices to perform a comprehensive *Legionella* risk assessment to identify potential hazards relating to exposure to *Legionella* bacteria from their water systems. In part, this involves decontaminating reusable instruments and daily flushing of dental unit water lines in primary care dental practices.

France

Regulations for *Legionella* surveillance and control in France were first introduced in 1997 for hot-water systems in healthcare facilities and more recently strengthened and extended to all public buildings (République Française 2005a,b, 2010a,b,c). The revised regulations set mandatory minimum temperatures and require *L. pneumophila* monitoring at defined points within hot-water systems, including outlets serving vulnerable persons.

In healthcare facilities, temperature is to be monitored daily or continuously at the hot-water heater outlet and at each return loop and weekly at service points. Temperatures must be greater than 55°C at hot-water heaters and greater than 50°C at distal points in the system. Furthermore, *L. pneumophila* concentrations by culture should be below the detection limit for all samples in hospitals with immunocompromised populations, otherwise immediate corrective measures must be taken. For non-immunocompromised patients, the threshold for immediate corrective measures is 1,000 CFU/L.

Environmental monitoring is also required in other public buildings with collective warm-water systems (e.g., hotels, nursing homes, senior residences, campsites, and tourist accommodation sites) and in some specific types of equipment such as cooling towers, atomizers used in public places, and thermal equipment. In public buildings with collective warm-water systems, the regulations state that the *L. pneumophila* concentration should not be greater than 1,000 CFU/L in hot water. If monitoring results are above the threshold, remedial actions should be taken immediately by the facility manager to protect the public and restore water quality. In spas, *L. pneumophila* should not be detected. In cooling towers, *L. pneumophila* concentration should not be greater than 1,000 CFU/L. When results come back higher than 100,000 CFU/L, the cooling tower should be stopped immediately, remedial actions should be taken by the facility manager, and the environmental authority must be informed. In thermal equipment supplied with natural mineral water, *L. pneumophila* and *Legionella* spp. should not be detected. In collective water-misting systems, *L. pneumophila* should not be detected. If the concentration is between 10 and 1,000 CFU/L, remedial actions should be started; when the concentration exceeds 1,000 CFU/L, the system must be stopped immediately.

Unlike other European countries, the French regulations are mainly based on environmental monitoring and specify what should be done by the facility manager in case of *Legionella* contamination or legionellosis cases, with less emphasis on a water management plan. Regional health and environmental agencies are the enforcement agencies for these regulations.

There has been no consolidation or analysis by the health ministry of environmental monitoring of *Legionella* for the many types of building hot-water systems and devices discussed above. Hence, it is not known whether the regulations have reduced the detection of *Legionella* in the environment. Nonetheless,

the number of legionellosis cases has stabilized (2.4 cases per 100,000 in 2017) and there have been no recent outbreaks (ECDC, 2019).

Australia

Regulations for management of *Legionella* in Australia are enforced by states or territories. As such, they vary in some details between jurisdictions, but they are all based on joint Australia and New Zealand standards (AS/NZS). These regulations are generally administered by state health agencies. Renewed interest in *Legionella* arose after a large outbreak at the Melbourne aquarium in 2000, attributed to a badly managed cooling tower. A hospital outbreak in 2013 led to increased testing at healthcare facilities. The regulations now focus on cooling towers and hot-water systems in buildings such as hospitals and other healthcare facilities. Spas are regulated under recreational water legislation (which is not discussed further).

Three national standards are enforced by the states and territories. The enHealth (2015) guidelines are for *Legionella* control during operation and maintenance of premise plumbing in healthcare and aged-care facilities. First published in 1991, the AS/NZS 3896 standard specifies sampling for *Legionella* spp. in water, while AS/NZS 3666 focuses on air handling and water systems of buildings, with an emphasis on cooling towers. These standards all have a general requirement for a risk management plan, as well as monitoring requirements for *Legionella* and other variables.

As per AS/NZS 3666, most jurisdictions in Australia require cooling towers to be registered, and they require dosing with a biocide, fitting of drift eliminators, regular servicing (monthly), and cleaning (every six months)—all of which are documented in a risk management plan. Some jurisdictions require monitoring of *Legionella*, with a range of required frequencies, and all testing is undertaken by standard culture methods. According to the regulations, a concentration of less than 10 CFU/mL (10,000 CFU/L) is considered to be non-detect. If samples exceed 10 CFU/mL twice consecutively, the building must be investigated, disinfected, and retested. If the concentration is greater than 1,000 CFU/mL (10^6 CFU/L) then decontamination is required. This concentration is not a science-based number; rather, it is based on what a well-maintained cooling tower can stay below. AS/NZS 3666 also specifies testing of heterotrophic plate counts (HPC), with thresholds of (1) less than 100,000 CFU/mL, (2) between 100,000 and 5 million CFU/mL, and (3) greater than 5 million CFU/mL. Some jurisdictions require notification of the public health regulator at lower *Legionella* or HPC levels, such as 10 CFU/mL.

The Australian regulations applicable to premise plumbing have taken two approaches. The first is to regulate only hot-water systems, allowing water delivered to bathrooms to be no more than 45°C (although they operate the hot-water system at 55°C to 60°C and have thermostatic mixing valves). The second is a risk assessment approach for all water systems, which is similar to ASHRAE 188 and other countries' regulations. The second approach is becoming more popular and stems from the enHealth guidelines for *Legionella* control in healthcare and aged-care facilities. There are no prioritized treatments of contaminated systems, but some hospitals do booster chlorination. As in the United States, this process can make the hospital a drinking water provider, but there is little oversight or regulatory burden involved. If a hospital has such a treatment system, it must hire professionals to manage the system. In general, the frequency of *Legionella* monitoring in premise plumbing is lower than in cooling towers.

There have not been any documented, quantitative impacts of the regulations on rates of Legionnaires' disease or environmental detection of *Legionella.* Australia has approximately 340 Legionnaires' disease cases reported each year, equally divided between *L. pneumophila* and *L. longbeachae* (see Figure 5-1 for cases in Queensland). *L. longbeachae* is more common in the south of Australia and generally associated

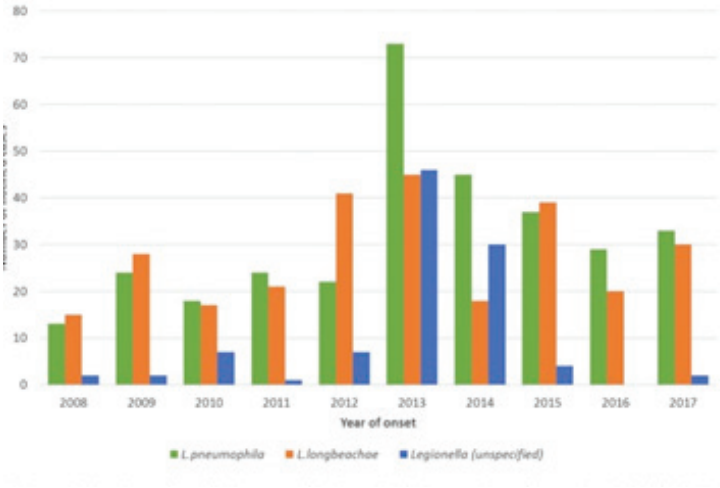

FIGURE 5-1 Number of notified cases of legionellosis by species, Queensland, 2008 to 2017.

SOURCE: Queensland Health (2018).

with soil and compost exposures. Cases have been relatively constant from 1997 to 2017, and the 2018 rate was 1.6 per 100,000 people. In Victoria, there was an increasing trend in Legionnaires' disease rates that peaked with the Melbourne aquarium outbreak. Program administrators state the regulations have helped to improve understanding of locations of higher risk and have led to better communication between building owners and operators (Cunliffe, 2018). In the next one to two years, *Legionella*-related regulations will be undergoing review in several jurisdictions and may be updated to include refinements in monitoring *Legionella*. In many ways, the Australian regulations are less strict than the Dutch regulations: their implementation varies by jurisdiction, some have not yet fully embraced the risk management paradigm, the suite of buildings covered is more limited (hotels are not covered unless they have a cooling tower), and the concentration thresholds for action are higher.

Canada

Compared with Europe, Canada has more limited and provincial regulations for *Legionella* management in buildings. After a Legionnaires' disease outbreak in Quebec City, the Public Services and Procurement Canada (PSPC) standard MD-15161 for control of *Legionella* in mechanical systems was created in 2013, covering the 360 buildings that are federally owned and managed by PSPC.· The PSPC portfolio is comprised mostly of office buildings across Canada. MD-15161 mandates minimum design, installation, operation, maintenance, and validation testing requirements to reduce the risks associated with *Legionella*. There are specific requirements for cooling towers; open water systems (e.g., ornamental water features); other heating, ventilation, and air conditioning (HVAC) components (e.g., humidifiers, drain pans); and domestic hot- and cold-water systems. Like other industry standards, MD-15161 requires the development of a site-specific plan (*Legionella* Bacteria Control Management Program) to identify susceptible systems, their risks and hazards, and site-specific considerations, and to ensure that appropriate mitigation measures and maintenance procedures are put in place. The plan must be reviewed and updated every five years, when there is a major change in procedures or replacement of equipment, or when *Legionella* testing has triggered an unscheduled disinfection of a system.

Temperature requirements are similar to many other countries, with greater than 60°C for hot-water heaters and greater than 50°C for distal points in hot-water systems (PWGSC, 2016). The monitoring required is specific to total *L. pneumophila*. The methods for cooling towers include monthly

culture testing for total *L. pneumophila*, with action levels of less than 10 CFU/mL (10^4 CFU/L); 10 to 1000 CFU/mL (10^5 to 10^6 CFU/L), which triggers cleaning and disinfection of the system; and more than 1,000 CFU/mL (10^6 CFU/L), which additionally requires the immediate cessation of any aerosol dispersal. qPCR for total *L. pneumophila* is conducted at system start-up, with action levels of less than 10 GE/mL; 10 to 100 GE/mL, which triggers a review of operations and treatment; and more than 100 GE/mL, which triggers cleaning and disinfection of the system. A residual oxidant test on a building's incoming water service is also required. To support implementation of the MD-15161 standard, a separate communications protocol has been developed that ensures timely, consistent, and appropriate communication to required stakeholders based on the maintenance action level triggered by the *Legionella* testing result.

Although MD-15161 is required of all the Crown-owned buildings managed by PSPC, the standard is starting to be adopted and applied by other federal departments that manage federal property. There is periodic reporting of compliance to MD-15161 to PSPC senior management and periodic auditing of facilities to ensure compliance to MD-15161. Nonconformities are identified through reporting and auditing and, when identified, a plan is put in place to rectify the problem.

PSPC started implementing MD-15161 in its portfolio in 2013 and started monitoring compliance in 2014, which has led to increased awareness of *Legionella* control from project inception to building operation and maintenance. There has also been a concomitant reduction in the number of positive results in cooling towers, as shown in Table 5-2, which provides data on the number of times unscheduled cleaning and disinfection of a cooling tower system was triggered by the *Legionella* testing required by MD-15161. There are approximately 200 cooling towers in the PSPC inventory.

TABLE 5-2 Effects of MD15161 on Cooling Tower Performance

Year	Number of times a cooling tower system required unscheduled cleaning and disinfection as per MD15161
2014	48
2015	27
2016	10
2017	11
2018	10

SOURCE: Data courtesy of Public Services and Procurement Canada.

Quebec Cooling Tower Regulations

Following the 2012 Quebec City Legionnaires' disease outbreak of 181 cases and 16 fatalities, the Régie du Bâtiment du Quebec (RBQ) introduced cooling tower regulations in 2013. Since then, all cooling towers in the Province of Quebec must be registered with provincial authorities. Cooling towers are defined as all open recirculating cooling systems, including cooling towers, fluid coolers, and evaporative condensers. The regulation requires a documented mechanical maintenance program and a documented water treatment program, including mandatory decontamination procedures. Mandatory monthly culture-based sampling for *L. pneumophila* was added in 2014 (RBQ, 2014). At levels less than 10^7 CFU/L, no action is required. At levels between 10^7 and 10^9 CFU/L, the cause of these high levels must be identified, and the owner must apply and confirm the efficacy of corrective measures. The health-based emergency

threshold is set at levels greater than 10^9 CFU/L, at which all equipment producing aerosols must be stopped and the owner must apply the emergency decontamination procedure and verify the efficacy of the corrective treatment. Compared to similar regulations in Europe, higher numbers were chosen as thresholds so as to not alarm people and to avoid the ambiguous implications of violating lower numbers.

Racine et al. (2019) collected and analyzed cooling tower treatment results from more than 323 evaporative cooling systems, along with corresponding *L. pneumophila* regulatory sampling results (n = 8,936) from July 2014 to June 2017. Water quality (i.e., hardness, alkalinity, chlorides, free and total chlorine, pH, conductivity) and treatment (corrosion and scale inhibitor, dispersant dosages) were determined. The analysis suggests that the introduction of the Quebec regulations raised the level of awareness and accountability in the management of cooling tower treatment programs, which led to a reduction in levels and incidences of *Legionella* positivity over the three-year period (see Figure 5-2). Interestingly, *L. pneumophila* control was most effective in systems using halogen-based biocides and on-line control of dosage. The study suggests that a regular review of the cooling tower treatment program and monitoring results, including *L. pneumophila* sampling, leads to a willingness to continue implementing control measures.

The Société Québécoise des Infrastructures (SQI) manages all cooling towers for provincial government buildings, which totals 39 buildings and 58 cooling towers. The buildings are located in different cities throughout the province and are fed by different water sources. Although all cooling towers are managed by SQI, each has a separate maintenance contract and, therefore, various disinfectants and frequencies of application. In total, 24 different biocides, both oxidants and non-oxidants, are used at various concentrations. Among cooling towers, 43 of 58 have two different disinfectants with continuous, daily, or weekly application. Only 13 of 58 cooling towers are operated year-round; the others are not operated during the winter months.

Monitoring results by culture and qPCR obtained between January 2014 and June 2017 were analyzed for temporal trends. Overall, a larger number of samples was positive by qPCR than by culture, as expected. As seen in Figure 5-3, the regulations were followed by a rapid decrease of positivity in cooling towers as measured by qPCR and culture. The small number of positive samples reveals significant progress in establishing efficient treatment regimes.

In 2016, case rates for Legionnaires' disease in Canada were reported at 0.87 per 100,000 people (Public Health Agency of Canada, 2016).

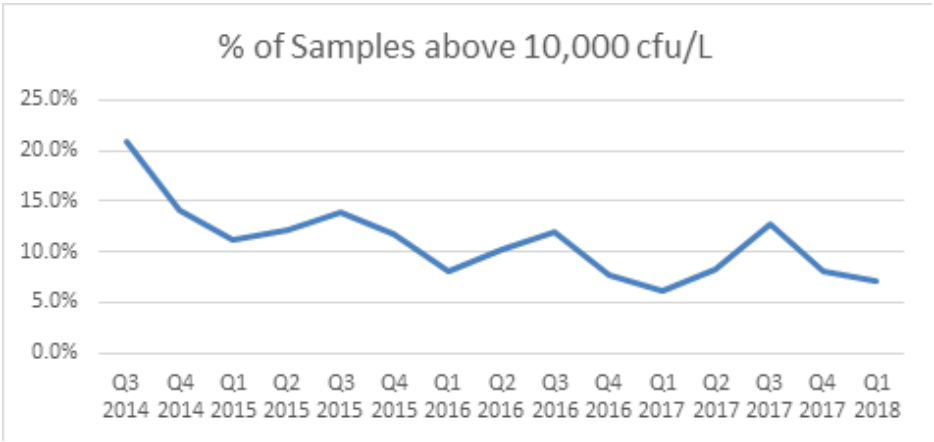

FIGURE 5-2 Percentage of Quebec cooling towers with *L. pneumophila* serogroups 1-14 concentrations exceeding 104 CFU/L over time.
SOURCE: Adapted from Racine et al. (2019).

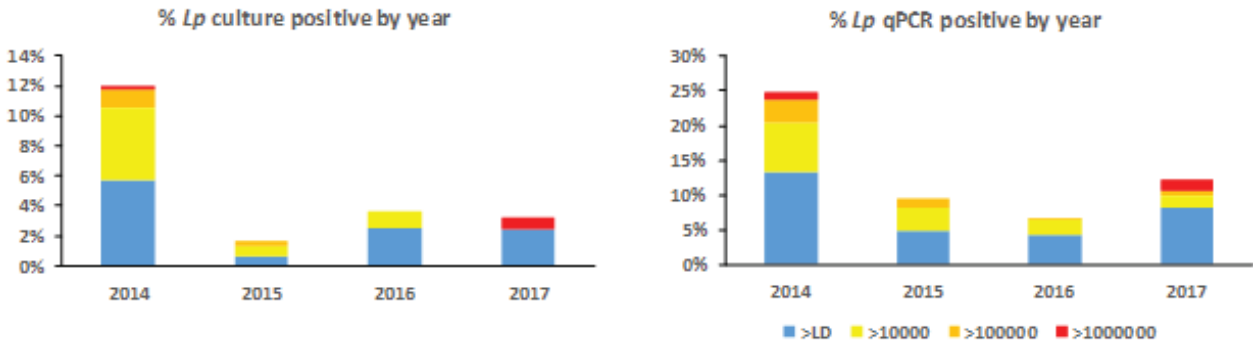

FIGURE 5-3 Percentage of cooling towers testing positive for *Legionella* by culture or qPCR, 2014-2017. Colors indicate samples above certain concentration thresholds.
SOURCE: Created using data from SQI.

Table 5-3 summarizes the above information on international laws and regulations governing *Legionella* management in water systems. The table illustrates the ubiquitous presence of temperature control as a control strategy for *Legionella* in buildings, as well as a requirement for monitoring of *Legionella* in building water systems—with the threshold for taking action between 10^3 and 10^4 CFU/L. This is consistent with Van Kenhove et al. (2018) who analyzed regulations in a much larger suite of countries and found that maintaining temperatures above 60°C and below 25°C, as well as monitoring of critical points, were common principles among the regulations.

For most of the countries, it is too early to make conclusions about the impact of regulations on rates of legionellosis, although there is evidence that the regulations are leading to declines in environmental detection of *Legionella*. Both the German building water system and Canadian government and Quebec cooling tower regulations have coincided with declines in the frequency of detection of *Legionella*. Lam et al. (2011) suggest that enactment of Environmental Public Health Regulations for cooling towers and water fountains in 2001 may have contributed to the decline of Legionnaires' disease cases in Singapore. The percentage of cooling towers testing positive for *Legionella* decreased from 58 percent during 2000–2002 to 13.7 percent during 2004–2008. While these examples do not provide direct evidence that regulations can decrease disease rates, they suggest that making *Legionella* control a priority is useful public policy.

In almost all cases, regulations for *Legionella* monitoring and control in Europe and elsewhere stemmed from a large outbreak. Prior to the existence of the regulations, there were few if any monitoring data, making the background level of *Legionella* in building water systems impossible to determine. The regulations have led to increased recognition of Legionnaires' disease, which may be contributing to increasing rates observed in the past few years. As more monitoring data are collected and analyzed, it is anticipated that the role of these regulations in preventing disease will become clearer.

STEPS FORWARD FOR U.S. *LEGIONELLA* MANAGEMENT

In the United States, there are no national regulations or guidance documents similar to those described in Table 5-3 for other countries, despite the 8,453 reported cases of Legionnaires' disease in the United States in 2018 (or 2.29 cases per 100,000, which is likely a gross underestimation of the actual burden—see Chapter 3). Furthermore, even among the directives, regulations, and guidance documents

TABLE 5-3 International Laws and Regulations regarding *Legionella* Management in Building Water Systems

Country	*Regulation/Standard/ Guideline*	*Buildings/Devices Covered*	*Risk Management Plan Required?*	*Preferred Treatment*	*Monitoring Thresholds (All Converted To CFU/L)*	*Enforcement/ Implementation*
Netherlands	1. Drinking Water Act (2011) 2. Hygiene and Safety Act (2000) 3. Safety at Work Act (2007) 4. Environmental Protection Act (2010)	1. Priority premises, which are basically large buildings 2. Swimming and bathing facilities 4. Wet cooling towers	Yes	Temperature control, flushing, UV, filtration	< 100 CFU/L, no action > 1,000 CFU/L, take response actions	National. Implementation of the Acts is increasing, but not yet 100%. Enforcement is via inspections, fines, possible facility closure
Germany	1. Protection against Infection Act 2. Emissions Control Act (2017)	1. Large building premise plumbing, swimming pools, bathing water, WWTPs 2. Cooling towers	Yes	None, though temperature control and avoiding stagnation evident in codes	> 1,000 CFU/L, take response action	National. Very high compliance. Small cooling towers slowly coming under regulation.
England	1. Health and Safety at Work Act, L8 regulations (2013 4th edition) 2. Health and Social Care Act (2008) 3. Notification of Cooling Towers and Evaporative Condensers Regulations (1992)	1. Evaporative cooling systems, hot- and cold-water systems, spa/ pool systems 2. Healthcare facilities 3. Cooling tower registration	Yes	Temperature control, biocides	< 100 CFU/L, no action 100-1000 CFU/L, take response actions > 1,000 CFU/L, alarm bell	England only. Enforced by inspectors, but they can do no preventative work.

TABLE 5-3 Continued

France	Regulations began in 2002 (République Française 2005a,b, 2010a,b,c)	Buildings except private residences, cooling towers	Only for cooling towers at this time	None apparent	< 1,000 CFU/L target for public facilities < 100 CFU/L target for prevention of nosocomial infections < 50 CFU/L target for hospitalized at-risk patients	Regional health and environmental agencies enforce the regulations
Australia	1. enHealth guidelines (2015) 2. Standards AS/NZS 3666 (2002, 2011) AS/NZS 3896 (2008, 2017)	1. Premise plumbing in healthcare and aged-care facilities 2. Cooling towers	Yes for cooling towers; becoming more risk-based for buildings	1. Temperature control, but broadening. 2. Biocides	< 10^4 CFU/L, no action > 10^6 CFU/L, take response actions	Varies by state and province
Canada	Standard MD-15161 (2013)	Cooling towers, open water systems, HVAC components, and hot- and cold-water systems in 360 buildings	Yes	None	< 10^4 CFU/L, no action > 10^6 CFU/L, take response actions	Oversight from Public Services and Procurement Canada, who does reporting and auditing
Quebec Cooling Tower Regulations	Régie du Bâtiment RBQ (2014)	Cooling towers	Yes	Biocides	< 10^7 CFU/L, no action ≥ 10^7 to < 10^9 CFU/L, take response actions ≥ 10^9 CFU/L, alarm bell	Oversight from Société Québécoise des Infrastructures

that extend to U.S. buildings, guidelines for interpreting *Legionella* monitoring data are lacking because of uncertainties over how to respond to certain values. These uncertainties have effectively hindered water utilities and building managers from testing for *Legionella*. Guidelines on routine environmental testing for *Legionella* vary among different documents:

- AIHA (2015) recommends that *Legionella* testing be conducted for validation of the water management plan or as part of an outbreak investigation. The goal is to have no positive samples, but corrective actions are required if samples have more than 10^3 CFU/L (see Table 5-1).
- ASHRAE 188 recommends that the team responsible for developing and implementing the building's risk management plan for *Legionella* control decide whether *Legionella* testing should be conducted. CMS (2017) requires that all healthcare facilities be consistent with ASHRAE 188.
- DVA (2014a) recommends routine environmental testing for *Legionella* in VHA facilities with prescribed actions required for any positive *Legionella* sample, regardless of the concentration.
- NSFI 453 requires monitoring for *Legionella* in cooling towers. *Legionella* is to be maintained at less than 10^4 CFU/L and investigations and corrective actions taken if levels are higher.
- The New York State regulations require hospitals and healthcare facilities to monitor their potable water systems for culturable *Legionella* and institute control measures and notify authorities when 30 percent or more of the samples contain *Legionella* spp.

Only the New York State regulations are enforced by law and impose civil and criminal penalties. The VHA guidelines are internally mandated, and the policy outlines specific responsibilities for various individuals. The CMS memo (2017) requires that all hospitals and healthcare facilities receiving Medicare or Medicaid funds have a water management plan or potentially lose funding (e.g., be "at risk of citation for non-compliance with the CMS Conditions of Participation"). Thus, all VHA facilities along with cooling towers and healthcare facilities in New York State are currently required to implement water management plans and monitor for *Legionella*. The CMS requirements, which cover hospitals and nursing homes nationwide, do not require monitoring for *Legionella* because ASHRAE 188 leaves the decision to the water management team. All other buildings and private residences are formally protected from *Legionella* only through the application of building and plumbing codes, and then only at discrete points in time when the codes are enforced. **There is a need for more uniform protection of public health from *Legionella* in hospitals and healthcare facilities, cooling towers, and building water systems across the country.** The following recommendations are made to develop a more comprehensive policy for *Legionella* management in the United States.

Recommendation 1. Expand the CMS Memorandum to Require Monitoring for *Legionella* in Environmental Water Samples

The CMS memo, which has high enforceability, requires that hospitals and long-term care facilities develop and implement water management plans. This memorandum has appropriately targeted buildings in which the mortality rates of Legionnaires' disease are high because of vulnerable patient populations, and it has increased awareness within healthcare organizations and provided needed focus on waterborne pathogens (e.g., *Legionella*). Routine quantitative *Legionella* monitoring programs would enable these institutions to assess the effectiveness of their water management programs. All healthcare organizations should have the capacity and expertise to use these data proactively to help limit nosocomial legionellosis. Such enhanced data collection from within hospital systems structures could help

to refine the data thresholds needed for prevention. This emphasis on monitoring is supported by the international regulations discussed above, by the VHA Directive and the New York State regulations, by AIHA (2015), and by the recent position paper from the America Water Technologies (AWT, 2019). The latter states that "*Legionella* testing is the only direct or 'active' way (currently) to validate program effectiveness, short of proving there is no Legionnaires' disease associated with the water management plan systems." Such an effort would also require strengthening of environmental laboratory capacity to process, evaluate, and interpret results from water samples, and it would necessitate documentation of laboratory proficiency. See below for a discussion of how to respond to the *Legionella* monitoring data that would stem from enforcement of an updated memo.

Recommendation 2. Register and Monitor Cooling Towers

Regulations and guidelines requiring the registration of cooling towers provide a demonstrable public health benefit with minimal regulatory burden to building owners and managers. Cooling tower registries enable a rapid public health response to community clusters of legionellosis cases, including timely remediation of possible sources of infection, and they can also be used to assess the contribution of cooling towers to overall disease incidence. In addition, regulations requiring ongoing *Legionella* monitoring of cooling towers have been shown to reduce cooling tower colonization rates in several jurisdictions where they have been implemented (e.g., Quebec Cooling Tower Regulations, Lam et al., 2011; Garland, Texas, Whitney et al., 2017). Unless the location of cooling towers is known, it is impossible to react quickly to identify the source of an outbreak or to assess the overall contribution of cooling towers to the occurrence of Legionnaires' disease.

Recommendation 3. Require Water Management Plans for All Public Buildings

The standard of care specified for water management plans (see the seven bullets on page 256) should be considered best management practice for all public buildings, including hotels, businesses, schools, apartments, and government buildings. This would extend the spirit of the Safe Drinking Water Act (particularly when building water treatment is used) into building water systems not covered by the CMS memo. The recommendation here is to make water management plans a requirement for all public buildings. This requirement would target the buildings likely to be sources of sporadic legionellosis, which constitutes 96 percent of all cases (see Chapter 3). ASHRAE 188, AIHA (2015), and other guidance documents are available to help create a water management plan that can meet this requirement. Derivative products that provide details for specific building types and devices, much like the CDC Toolkit has done for healthcare facilities, would be useful to help implement this recommendation.

The team developing and implementing the water management plan would outline the operation, monitoring, and maintenance of building water treatment systems using licensed and trained individuals to collect and retain appropriate records and monitoring data. (The required training of professionals is discussed in a subsequent section.) Similar to other public health certifications, application records and monitoring results could be required only on inspection, which would minimize unnecessary compliance reporting activities. Should inspections reveal serious defects in the execution of the building water management plans, penalties, including loss of certification and public notices on building entrances, could be used.

Ideally, this requirement would be codified by either local jurisdictions with authority (e.g., build-

ing inspectors) or state authorities (e.g., departments of environmental protection or health). Once codified, the requirements could be supported by insurance companies—that is, without a water management plan, a building would not qualify for insurance.

Recommendation 4. Require a Temperature of 60°C (140°F) at Hot-Water Heaters and 55°C (131°F) to Distal Points

Optimal operating temperatures at critical points in the hot-water system are based on an international consensus that maintaining minimum temperatures across the different parts of a hot-water system is the first barrier to implement to restrict *Legionella* growth, even if continuous disinfection is present. Typically, the requirements include maintaining temperatures of greater than 60°C at the water heater and in reservoirs, greater than 55°C in the return loop, and ensuring that distal points reach a minimum temperature of 55°C within one minute of use. Monitoring temperature at the distal point of hot-water systems would be necessary to verify that this requirement is being met. As discussed in Chapter 4 and shown in Table 4-3, 55°C carries a scalding risk that can be reduced with the use of thermal mixing values in buildings with sensitive populations.

The countries previously discussed in this chapter all include objectives for optimal operating temperatures in the hot-water system; design and operational specifications for water heaters, storage, and recirculation systems; and recommendations for maximum temperature at distal points. In England, France, Germany, and the Netherlands, periodic monitoring of *Legionella* and temperature is mandatory, with a frequency varying from continuous to weekly or annually depending on the parameters, the risk classification, and the location of the point of use. The change recommended here would align the United States with these countries.

These temperature requirements could be codified by changing building and plumbing codes. However, to impact healthcare facilities more immediately, this requirement could be instituted by modifying the CMS memo. It is also possible to incorporate these requirements into ASHRAE or other guidance; ASHRAE 12-2000 section 4.1.6 already states: "In a high-risk system, cold water should be below 20°C, hot water should be stored above 60°C and returned and circulated at above 51°C." A required temperature at distal points could be inserted into ASHRAE 12-2000 as it undergoes revision in the near future.

Recommendation 5. Require a Minimum Disinfectant Residual Throughout Public Water Systems and Concomitant Monitoring for *Legionella*

It is important that water entering domestic and public buildings be of the highest quality possible. Currently, federal regulations allow for no disinfectant residual in 5 percent of the measurements in distribution systems that use surface water as a source (EPA, 1989). Federal law does not require groundwater systems to maintain any disinfectant residual within their distribution systems. *L. pneumophila* has been shown to grow in public water systems in which chlorine residuals were less than 0.1 mg/L (LeChevallier, 2019b). EPA should require a minimum disinfectant residual throughout public water systems and validate treatment performance by routine monitoring for *L. pneumophila* from sampling sites representative of the distribution system. Monitoring could focus on warm-water conditions and be triggered once water temperatures consistently exceed 20°C although some detection may occur above 15°C (LeChevallier, 2019b). Corrective actions should be taken once the concentration of *L. pneumophila,* or the frequency of occurrence, exceeds specific trigger levels consistent with science-based and develop-

ing guidelines and risk assessments.

Legionella has been on the EPA Candidate Contaminant List for the past ten years, which means that the agency should be evaluating methods to determine the occurrence of the organism in public water systems. However, no action has been taken because the agency is unsure how to interpret or communicate positive *Legionella* results in treated water supplies. The development of guidelines for interpreting monitoring data, outlined below, could provide the framework for including *Legionella* in the next round of unregulated contaminant monitoring (UCMR 5) scheduled to begin in 2022.[7] However, utilities need not wait until EPA conducts the UCMR monitoring; they could start collecting this information now and set internal targets for distribution system optimization.

Develop Guidelines for Interpretation of *Legionella* Monitoring Data Using a Risk-Based Framework

The lack of clear guidelines from the EPA or the CDC has hampered collection by building owners and water utilities of *Legionella* occurrence data over fears that a single detection could trigger onerous remediation requirements by public health and environmental regulators. In fact, monitoring for *Legionella* in the absence of any cases of Legionnaires' disease has not been recommended by the CDC.[8] ASHRAE 188 does not require *Legionella* monitoring as part of the building water management plan. Yet, three of the five recommendations above incorporate monitoring of *Legionella* in building water systems, which will require a framework in order to interpret the data.

There are at least two contexts in which monitoring results might be used to make decisions. The first is to detect the imminent threat of an outbreak of legionellosis based on monitoring and quantifying *Legionella* at points close to end uses. The Committee's review of available data, presented in Chapter 3, indicates that when a series of measurements show *Legionella* levels in excess of 5×10^4 CFU/L, there is an imminent risk of an outbreak occurring. At this point, urgent action to discontinue exposure and implement a remediation plan is appropriate. For individuals who are highly susceptible (e.g., immunocompromised patients in hospital and healthcare settings), or where a greater safety factor is desired, it would be appropriate to reduce this action level multifold. The 5×10^4 CFU/L action level, which would be relevant to all building water systems, falls between the lower thresholds found in the European countries and the higher thresholds found in Australia and Canada, as discussed above. This action level is also consistent with the AIHA (2015) guidelines for all building water systems (except cooling towers). Notably, none of the numeric thresholds from these other regulations or guidances stem from any risk-based assessment but rather from practicality and expert judgement.

The second context for data-based decisions is when there is a routine program of quantifying *L. pneumophila*[9] to determine that the risk of legionellosis is acceptably low. In this case, quantitative microbial risk assessment (QMRA) can be used to develop routine operational targets for different types of building water systems. For example, based on an analysis of acceptable risk levels, and using a 10^{-6} DALY/person-year as a target (Hamilton et al., 2019; see Chapter 3) single sample water concentrations of *L. pneumophila* of less than 1,060 CFU/L, 8,840 CFU/L, and 14.4 CFU/L in faucets, toilets, and showers, respectively, could be regarded as acceptable. In practice, one would use the most stringent number, which would be the 10^3 CFU/L for buildings with just toilets and sinks and 10 CFU/L for buildings with showers. For buildings with showers, the number 10 CFU/L equates to 1 CFU/100 mL, which is the detection limit of current monitoring approaches for many laboratories (ISO, 2017). From a risk

[7] See https://www.epa.gov/dwucmr.

[8] See https://www.cdc.gov/legionella/health-depts/ashrae-faqs.html.

[9] *L. pneumophila* is indicated here, rather than *Legionella* spp., only because the current dose-response relationship is for *L. pneumophila*.

protection standpoint, this value substantiates the very reasonable goal of having only non-detects in a hospital environment (see Box 3-8).

Cooling towers have been more difficult to model with QMRA because the exposure pathway from the water column via aerosols to individuals is much more complex and poorly described. Further research using dispersion models will be necessary to further refine *L. pneumophila* concentrations that correspond to specific risk values from cooling towers.

Train and Educate Those in Relevant Disciplines and Occupations Who Are Responsible For the Safety of Water Systems

The five recommendations above will require training and education on legionellosis and on the prevention and control of *Legionella* amplification in water systems for a variety of professionals, including building owners and operators, engineering consultants, clinicians and epidemiologists, laboratory technicians, inspectors, and architects, among others. The medical community curriculum should be expanded to include all aspects of diagnosing and treating legionellosis in the most effective manner. This education should also include sufficient aspects of water management to effectively educate patients on inherent risks within their homes that may require attention when they are released from the hospital. Education and training are also needed for those designing water systems, for those overseeing municipal water supplies, for those developing and implementing plumbing codes, and for those in government responsible for the safety of buildings, cooling towers, and the potable water supply. Building operators and their staff require a basic understanding of *Legionella*, their ecology and growth conditions, as well as sampling collection techniques for *Legionella*. Initial training, as well as continuing education, should be required for individuals responsible for maintenance of water operations and premise plumbing. Box 5-1 discusses two recent training programs specific to *Legionella*.

In addition to being trained, those managing building water systems should be part of the water management program team and they should be certified to operate any water treatment system. This way, the operator's certification can be held responsible, and possibly lost, if the building water systems are not properly maintained (in addition to any possible criminal liabilities for failure to act upon their training and certification). The certification should also require on-going educational training. Multiple entities could, and in some cases have, developed certification programs.

Disconnect Between Experts in Design, Construction, Operations, and Materials

Building water system design is often compromised because water management plans and their successful implementation are currently not understood across the building professional landscape. Even in cases where the building owner is very specific on a plan or a design to prevent waterborne pathogens, the building industry has multiple fragmented or siloed participants who preclude successful *Legionella* prevention. Without continuous, almost daily, interaction with all participants in the building and construction process, the likelihood of achieving the desired result can be marginal.

One challenge to progress is that the largest portion of tacit knowledge resides with the building owner and building operations professionals. In many instances, barriers to success can be associated with a general lack of understanding about water management plans by other professionals represented across the paradigm of building construction—engineers and architects who design the building, construction managers and general contractors who hire and manage the trades, and the trade groups that build the building. Although building commissioning professionals add to oversight on behalf of the own-

BOX 5-1
Recent Training Modules Relevant to Control of *Legionella*

The CDC in conjunction with the Western Region Public Health Training Center at the University of Arizona recently launched a free on-line training program titled Preventing Legionnaires' Disease: A Training on *Legionella* Water Management Programs (see https://www.cdc.gov/nceh/ehs/elearn/ prevent-LD-training.html). The training provides an opportunity to gain knowledge on how to reduce *Legionella* risk via water management programs, similar to the approach in the CDC's tool kit (CDC, 2017) developed following the release of ASHRAE 188. Training helps build common language among professionals utilizing water management programs through case study templates and other practical resources. The program is designed for professionals involved in water management programs including public health professionals, infection preventionists, building managers, maintenance and engineering staff, safety officers, and equipment and water treatment suppliers, as well as consultants. The training is relevant to building water systems in hospitals, retirement homes and long-term care facilities, hotels, apartments, and other buildings, as well as other devices that may need a water management program even if the building does not, such as cooling towers, decorative water features, hot tubs, and misters.

The American Society of Sanitary Engineering (ASSE) offers training and certification on infection control and water quality. Unlike the CDC training, the ASSE training is trade specific and requires prerequisite training and experience to take the certification coursework. The ASSE/IAPMO/ANSI Series 12000-2018, Professional Qualifications Standard for Infection Control Risk Assessment for All Building Systems, defines general knowledge requirements for developing and implementing water systems risk management programs and sets minimum criteria for training and certifying employers, plumbers, pipefitters, HVAC technicians, and sprinkler fitters. Different aspects of the series 12000 certification are currently provided through certified instructors and 18 union-based trade schools. The certification program provides general knowledge of pathogens, biohazards, and infectious diseases for plumbing, piping, and mechanical systems workers, or any individual who has the potential for exposure to pathogens, biohazards, or other potentially infectious material. Additional parts of the series provide general knowledge about contamination and infection prevention procedures to protect facility occupants and operations. The higher level (12060 to 12063) certifications build on the lower series designations as prerequisites and provide additional general knowledge on developing and implementing water systems risk management programs for plumbing, mechanical, and water-based fire protection systems (see http://www.assewebstore.com/asse-iapmo-ansi-series-12000-2018-download/). As more and more facilities, particularly healthcare facilities, begin requiring training and certification in infection control, ASSE 12000 series certifications will prove to be valuable credentials. The certifications address the need for construction and maintenance personnel to become proficient in identifying and managing potential situations where they may be exposed to bloodborne, waterborne, and airborne pathogens. These certifications also cover the responsibility of personnel to protect building occupants and operations from pathogens and hazards, especially in healthcare facilities. These certifications allow recipients to comply with standards and all piped systems that currently reside in all occupied buildings (see http://www.asse-plumbing.org/asse/personnel-certification/12000).

er and the operators, unless these individuals are specifically educated and understand the relevance of water safety, their value in preventing waterborne pathogens via effective design and plans is negligible. In most cases, construction design and plans to specifically reduce the incidence of waterborne pathogens come at a higher cost than standard construction methods.

Building design and construction professionals are further segregated by building type, with commercial building construction being highly specialized. Each of the major categories of building type (e.g., commercial offices, factories, warehouses, schools, retail, entertainment, data facilities, healthcare) has specific subgroups with special needs and requirements. Requirements regarding aesthetics and specific use are developed by architects and design professionals while infrastructure systems are developed by mechanical and design engineers. The design professionals may be highly specialized and segregated, whereas the building trades (e.g., carpenters, plumbers, electricians) are common to all. Training and certification are mechanisms for overcoming this inherent segregation, such that all professionals involved in building design, construction, and operation have an equivalent *Legionella* knowledge base.

Implementation and Cost Considerations

Although ordered 1 to 5, the five recommendations discussed above are not prioritized. In fact, accomplishing any one of them would lead to important legionellosis risk reduction, with the effect being cumulative as more recommendations are instituted. The five goals differ substantially in the necessary implementation schedule, what entities would provide oversight, their cost, and what other capacities need to be in place to support them.

Adding a requirement for monitoring within the CMS memo (Recommendation #1) could be expanded relatively quickly. Meeting this goal would entail an initial assessment of the current laboratory capacity for testing and an estimate of the number of samples that might be added to the annual work load. Most labs that are ELITE certified (see Chapter 3) can accurately identify *Legionella* bacteria, but quantification needs to be improved and should be made part of every proficiency testing program before *Legionella* monitoring can occur on a large scale. Once capacity is adequate, the implementation of monitoring could be accomplished in phases over the next three years, based on populations at risk, with oversight from the U.S. Department of Health and Human Services. Examples of hospitals already conducting such monitoring (e.g., see Box 3-8) will provide case studies and protocols.

Registering cooling towers (Recommendation #2) could follow New York's lead, and is most appropriately coordinated at the state level, including state and city departments of health or departments of environmental protection. Any state with an outbreak or some threshold number of legionellosis cases could be prioritized, with the objective of all states meeting the requirement over a five-year time frame.

The requirement to develop water management plans in public buildings (Recommendation #3) may be the most complex and costly recommendation by the Committee, as it would require that building owners become trained and incentivized to conform via governance structures. Hopefully, with oversight from the states and assistance from OSHA, this recommendation would be implemented over the next decade by those with expertise in engineering and building water quality. Key business chains, such as hotels, could take national leadership roles, and insurance companies could require water management plans before insuring buildings.

Increasing temperature requirements (Recommendation #4) will require education and oversight via local jurisdictions and plumbing codes. Given the three-year cycles inherent to changing most plumbing codes, this requirement could not be expected to happen quickly, nor would the requirements reach a broad swath of at-risk buildings, given the limited time frames of code enforcement. However, if the

temperature requirement was codified in the CMS memo, then much more rapid risk reduction, based on elevating building water temperatures, could occur.

And finally, water utilities will be responsible for managing, monitoring, and addressing disinfectant residuals and new requirements for *Legionella* monitoring in drinking water distribution systems (Recommendation #5). EPA should be involved, and ultimately a few of the larger utilities will likely provide guidance on best practices to implement the strategy. It is anticipated that implementation of the recommendation would take five to ten years.

Clearly, capacity building will be needed, including training programs (discussed previously), accreditation and certification of laboratory programs, and education of qualified and proficient consultants. In addition, the many work streams that will be created must be devoid of conflicts of interest, which is challenging given that many professionals are linked to a specific mitigation strategy or promote a specific product or service. For example, any individual, vendor, or contractor who develops a water management plan or provides a mitigation modality should not be responsible for monitoring the effectiveness of the plan.

None of the five recommendations for *Legionella* risk reduction are without implementation costs, although these are currently unknown. For this reason, cost-benefit analyses conducted by the appropriate responsible entities (such as water utilities for Recommendation #5) would be worthwhile to better understand the costs involved in carrying out the individual recommendations. Some costs will likely be passed from building and cooling tower owners onto building occupants. It may be possible, however, to meet some of the financial needs imposed by the five recommendations by adding a fee onto user water bills. Such a fee is similar in nature to the fees charged or collected by other utilities and subsequently used to subsidize improvements, conduct research, or to enhance existing utility processes. The fees could also support education and training for individuals to become competent professionals in waterborne pathogens and disease mitigation and prevention.

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Acronyms

AIHA	American Industrial Hygiene Association
ANSI	American National Standards Institute
AOC	assimilable organic carbon
APHA	American Public Health Association
ASHRAE	American Society of Heating, Refrigerating and Air-Conditioning Engineers
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
AWWA	American Water Works Association
BAL	bronchoalveolar
BCYE	buffered charcoal yeast extract
BDOC	biodegradable dissolved organic carbon
BMP	best management practice
CAPNETZ	Competence Network for Community-Acquired Pneumonia
CCL	Candidate Contaminant List
CCR	consumer confidence reports
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFU	Colony forming units
CMS	Centers for Medicare & Medicaid Services
CSTE	Council of State and Territorial Epidemiologists
CT	computed tomography
DALY	Disability-adjusted life years
DFA	direct fluorescent antibody testing
DNA	deoxyribonucleic acid
DOHMH	Department of Health and Mental Hygiene
DVA	Department of Veterans Affairs
DWSD	Detroit Water and Sewerage Department
ECDC	European Centers for Disease Control
EEA	European Economic Area

ELAP	NYS Environmental Laboratory Approval Program
ELITE	Environmental *Legionella* Isolation Techniques Evaluation
EMA	ethidium monoazide
EPA	Environmental Protection Agency
EU	European Union
EWGLI	European Working Group for *Legionella* Infections
FDA	Food and Drug Administration
FY	fiscal year
GAC	granular activated carbon
GC	gene copy
GCHD	Genesee County Health Department
HHS	Department of Health and Human Services
HIV	human immunodeficiency virus
HPC	heterotrophic plate count
HVAC	heating, ventilation, and air conditioning
IAPMO	International Association of Plumbing and Mechanical Officials
ICU	intensive care unit
IHC	immunohistochemistry
ISO	International Organization for Standardization
LD	Legionnaires' disease
LEED	Leadership in Energy and Environmental Design
Lp	*Legionella pneumophila*
LPS	lipopolysaccharide
MCLG	maximum contaminant level goal
MIF	mature infectious form
MIP	macrophage infectivity potentiator
MPN	most-probable-number
NIAID	National Institute of Allergy and Infectious Diseases
NNDSS	National Notifiable Disease Surveillance System
NOM	natural organic matter
NORS	National Outbreak Reporting System
NSF	National Science Foundation
NSFI	National Sanitation Foundation International
NYC	New York City
NYS	New York State
PCR	polymerase chain reaction
PEX	cross-linked polyethylene
PFGE	pulsed field gel electrophoresis
PHB	polyhydroxybutyrate
PMA	propodium monoazide
POE	point of entry
POU	point of use
PSPC	Public Service and Procurement Canada
PVC	polyvinyl chloride
PWGSC	Public Works and Government Services Canada
QMRA	quantitative microbial risk assessment

qPCR	quantitative PCR
RNA	ribonucleic acid
RO	reverse osmosis
SLDSS	Supplemental Legionnaires' Disease Surveillance System
SQI	Société Québécoise des Infrastructures
ST	sequence type
S/V	surface-to-volume
SDWA	Safe Drinking Water Act
SWTR	Surface Water Treatment Rule
TDS	total dissolved solids
TLR	toll-like receptors
TNTC	too numerous to count
TOC	total organic carbon
UAT	urinary antigen test
UCMR	Unregulated Contaminant Monitoring Rule
USGBC	U.S. Green Building Council
UV	ultraviolet
VBNC	viable-but-not-culturable
VHA	Veterans Health Administration
VSP	Vessel Sanitation Program
WHO	World Health Organization

Appendix
Biographical Sketches of Committee Members and Staff

JOAN B. ROSE, (NAE), Chair, is a professor at Michigan State University and holds the Homer Nowlin Chair in Water Research. She serves as the co-director of the Center for Advancing Microbial Risk Assessment, which addresses evidence-based risk assessments for management of waterborne pathogens. Dr. Rose is an international expert in water microbiology, water quality, and public health safety, and has published more than 300 manuscripts. For more than 20 years, she has been involved in drinking-water investigations of waterborne outbreaks and is well known for her work on the waterborne outbreak of Cryptosporidium in Milwaukee. She is a pioneer in the emerging science of viral metagenomics—sequencing virus DNA in water sources, discharges, and shipping ballast using next generation high-throughput technology. Her global activity includes investigation of waterborne disease outbreaks and the study of water supplies, treatment, and reclamation. Her applied research interests include study of microbial pathogens in recreational waters and climatic factors impacting water quality. Dr. Rose recently won the Stockholm Water Prize and is a member of the National Academy of Engineering. She is a member of the International Joint Commission, Health Professionals Advisory Board. She has served on numerous boards and committees of the National Academies of Sciences, Engineering, and Medicine and is currently a member of the Board on Environmental Studies and Toxicology. Dr. Rose earned her B.Sc. from the University of Arizona, her M.S. from the University of Wyoming, and her Ph.D. from the University of Arizona, all in microbiology.

NICHOLAS J. ASHBOLT is the Alberta Innovates–Health Solutions Translational Health Chair in Infectious Diseases at the University of Alberta School of Public Health. He was previously a senior research microbiologist in the Office of Research and Development at EPA. Before that, he was the head of the School of Civil and Environmental Engineering at the University of New South Wales, Sydney, where he was a professor and deputy director of the Centre for Water and Waste Technology. He has also been the principal scientist at the Sydney Water Corporation, Australia. Since 2000 he has specifically worked on detecting *Legionella* within piped water system biofilms, developed the first quantitative microbial risk assessment (QMRA) model to identify critical concentrations and prioritize research needs for future *Legionella* risk assessments and management, and is working on drinking water safety plans for *Legionella* in Alberta. Over the past 20 years, he has worked on joint Australian and European programs to develop methods to interpret pathogen data with the aid of QMRA within an urban water sustainability framework. This work has contributed to the risk-based approach adopted in the most recent Australian and

World Health Organization guidelines for recreational water use, drinking water, and water reuse. Dr. Ashbolt received his B.Ag.Sc. and his Ph.D. in microbiology from the University of Tasmania.

RUTH L. BERKELMAN (NAM) is the Rollins Professor Emerita in Epidemiology at Emory University and has held appointments in the departments of epidemiology, global health, and medicine, and the Emory Ethics Institute. She is an international expert in infectious diseases and public health policy, and has engaged on issues related to waterborne disease. She has investigated outbreaks, has conducted research, and has led policy discussions on the prevention and control of legionellosis. Before coming to Emory, she served in many positions, including the Deputy Director of the National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC). She retired as an Assistant Surgeon General after 20 years with the U.S. Public Health Service. Dr. Berkelman has served on various committees and boards including the HHS National Biodefense Science Board, the NRC Board of Life Sciences, and the Princeton University Board of Trustees. She is a member of the National Academy of Medicine and the American Academy of Microbiology, and is currently serving on the External Advisory Board for the College of Public Health and Health Professions, University of Florida. She holds an A.B. degree from Princeton University and an M.D. from Harvard Medical School. She is board certified in pediatrics and internal medicine.

BRUCE J. GUTELIUS is the medical director of the Enterics, Waterborne, and Health Education Unit at the Bureau of Communicable Disease within the New York City Department of Health and Mental Hygiene (DOHMH). Dr. Gutelius has more than ten years of experience in public health at the state and local levels, with expertise in infectious disease and chronic disease epidemiology and public policy. In his current role, he oversees disease surveillance and outbreak investigations for foodborne and waterborne diseases including the 200 to 400 cases of legionellosis that occur in New York City (NYC) each year. He has led the development of the NYC DOHMH's protocols for disease surveillance and response to legionellosis clusters, including standard approaches to data collection, analysis, and reporting; prioritization of interventions; interpretation of clinical and environmental testing; provision of logistical support; and development of communications materials for medical providers, building owners, elected officials, the media, and the public. He oversees the NYC DOHMH's ongoing collaboration with CDC to assess the effectiveness of NYC's cooling tower regulations in preventing environmental contamination and human disease related to *Legionella*. Dr. Gutelius received clinical training in internal medicine at the University of Rochester and in endocrinology, diabetes, and metabolism at the University of Pittsburgh. He holds a B.S. in biology from Oberlin College, an M.P.H. from the University of Pittsburgh, and an M.D. from Albany Medical College.

CHARLES N. HAAS is the L. D. Betz Professor of Environmental Engineering and head of the Department of Civil, Architectural, and Environmental Engineering at Drexel University, where he has been since 1991. He also has courtesy appointments in the Department of Emergency Medicine of the Drexel University College of Medicine and in the School of Public Health. He has served on the faculties of Rensselaer Polytechnic Institute and the Illinois Institute of Technology prior to joining Drexel. He co-directed the EPA–U.S. Department of Homeland Security (DHS) University Cooperative Center of Excellence's Center for Advancing Microbial Risk Assessment (CAMRA). He is a fellow of the American Academy for the Advancement of Science, the American Academy of Microbiology, the American Society of Civil Engineers, the Association of Environmental Engineering and Science Professors, the International Water Association, and the Society for Risk Analysis. He is a Board Certified Environmental Engineering Member by eminence of the American Academy of Environmental Engineers. For more than 35 years,

Dr. Haas has specialized in the assessment of risk from and control of human exposure to pathogenic microorganisms and, in particular, the treatment of water and wastewater to minimize microbial risk to human health. Dr. Haas has served on numerous committees of the National Academies of Sciences, Engineering, and Medicine and is a past member of the Water Science and Technology Board and the EPA Board of Scientific Counselors. He has worked on developing risk assessment models for *Legionella*, occurrence in engineered water systems, and disinfection in water systems. He received his B.S. in biology and his M.S. in environmental engineering from the Illinois Institute of Technology, and his Ph.D. in environmental engineering from the University of Illinois at Urbana-Champaign.

MARK W. LeCHEVALLIER is the principal and manager of Dr. Water Consulting, a part-time consulting business, after retiring from American Water at the end of 2017. Dr. LeChevallier received his B.S. and his M.S. in microbiology from Oregon State University, and his Ph.D. in microbiology from Montana State University. He has authored more than 300 research papers and has received awards from the American Water Works Association for outstanding contributions to the science of water treatment. Dr. LeChevallier was the recipient of the George Warren Fuller award in 1997 from the New Jersey section of the American Water Works Association, as well as the Abel Wolman Award in 2012 and the A.P. Black Award for research in 2015, both from the American Water Works Association. His research areas have included bacterial regrowth, disinfection of biofilms, corrosion, assimilable organic carbon measurement techniques, biological treatment, Mycobacterium, *Legionella*, microbial recovery and identification, modeling and impact of pressure transients on water quality, and detection, treatment, and survival of Giardia and Cryptosporidium. He is a fellow of the American Academy of Microbiology.

JOHN T. LETSON is vice president of plant operations at Memorial Sloan Kettering (MSK) Cancer Center. He is a facilities operations executive experienced in all aspects of organizational management, compliance, technical engineering, and operations as they apply to infrastructure, construction, and renovations in research and healthcare environments. He started at MSK in 1999 as manager of plant operations and held multiple positions before being promoted to vice president in 2013. He is now responsible for all plant and facilities operations and MSK skilled trade groups throughout the enterprise. Graduating from SUNY Maritime College with a B.E. in naval architecture and marine engineering, he worked for 17 years for a major oil company's marine transportation department, followed by five years in construction and property management. While at MSK, he earned an M.B.A. from Hagan School of Business at Iona College. He is a founding member of MSK Green Team–leading energy-related initiatives and supporting sustainability. Mr. Letson is the author of MSK's *Legionella* monitoring, prevention, and control policies, procedures, and plans.

STEVEN A. PERGAM is an associate member in both the Clinical Research Division and the Vaccine and Infectious Disease Division of the Fred Hutchinson Cancer Research Center. He is also an associate professor in the Department of Medicine, Division of Allergy and Infectious Diseases, at the University of Washington and an adjunct associate professor in the Department of Epidemiology, School of Public Health, University of Washington. He serves as the medical director of infection prevention and is an attending physician at the Seattle Cancer Care Alliance. Dr. Pergam focuses his research on the prevention and treatment of infections among immunocompromised patients and has expertise in infection prevention and hospital epidemiology among cancer and transplant patients. He has lectured, published, and mentored students on the prevalence and diagnosis of *Legionella* infections in this population. He serves on numerous national committees including the CDC's Advisory Committee on Immunization Practices and the National Comprehensive Cancer Network Committee on the Prevention and Treatment

of Infections in Cancer. He is an associate editor of Current Opinions in Infectious Disease and BMC Infectious Diseases. In 2014, he was elected to be a fellow of the Infectious Disease Society of America. He received his B.A. from Dartmouth, his M.D. from the University of Nebraska, his infectious diseases fellowship training at the University of Washington, and his M.P.H. from the University of Washington, School of Public Health.

MICHÈLE PRÉVOST is the Industrial Chair on Drinking Water of the National Science and Engineering Council of Canada at the Department of Civil Engineering of Polytechnique Montreal. Dr. Prévost's research has focused on source water protection, water treatment (including disinfection), and various aspects of distribution systems (e.g., lead control, biostability, pathogen regrowth, integrity and intrusion, data mining, and hydraulic and water quality modeling). Recently, she has directed the multi-university utility partnership initiative to reduce lead at the tap through a suite of laboratory, field, and epidemiological studies in Canada. She was a member of the technical advisory committee to the Walkerton Commission and presided over the Quebec RESEAU Advisory Committee on Drinking Water Regulations for 12 years. In 2016, Dr. Prévost received the A. P. Black Research Award of the American Water Works Association for outstanding research contributions to water science and water supply rendered over an appreciable period of time. In the past five years, Dr. Prévost has secured funding to expand collaborative research activities with healthcare facilities to assist them with emerging water quality issues caused by premise plumbing. She received her B.Sc. in renewable resources from McGill University, her M.A.Sc. in environmental and civil engineering from Ecole Polytechnique de Montréal, and her Ph.D. in civil engineering from Polytechnique Montréal.

AMY PRUDEN is the W. Thomas Rice Professor of Civil and Environmental Engineering at Virginia Tech. Her research focuses on bringing a microbial ecological perspective to understanding and advancing design and management of environmental systems. Dr. Pruden is a leading expert on water-based pathogens and antibiotic resistance. In 2012–2013, she led a Water Research Foundation expert workshop and report on Opportunistic Pathogens in Premise Plumbing: Epidemiology, Microbial Ecology, and Engineering Controls, in which a multi-stakeholder framework for public health protection was developed. In 2016, she co-organized a workshop sponsored by the Alfred P. Sloan Foundation at Emory University, titled "From Watersheds to Showerheads: Improving *Legionella* Risk Management in the 21st Century." Her current research focuses on how engineering design shapes the composition of the microbiome of tap water and implications for control and spread of *Legionella*, *Naegleria fowleri*, and antibiotic resistance genes. She has authored more than 100 peer-reviewed scientific journal articles including 30 papers in the previous five years focused on *Legionella* and other opportunistic pathogens. Dr. Pruden is the recipient of the Presidential Early Career Award in Science and Engineering and the Paul L. Busch Award for innovation in water research. She holds a B.S. in biology and a Ph.D. in environmental science, both from the University of Cincinnati.

MICHELE S. SWANSON is a professor of microbiology and immunology in the University of Michigan Medical School, where she is also the director of the Office of Postdoctoral Studies. Dr. Swanson's primary research interest is investigating the mechanisms that govern the innate and adaptive immune responses when macrophages ingest microbes, using *Legionella pneumophila* growth in macrophages as a model system. Currently, her laboratory is investigating whether changes in the chemistry of Flint, Michigan's water supply altered persistence or virulence of *Legionella pneumophila*. She is also investigating *Legionella* as part of two other projects—one on microbial water quality in domestic hot-water supply and recirculation systems and the other on enhanced disease surveillance and environmental monitoring.

She was previously a research fellow at the Howard Hughes Medical Institute, Tufts Medical School, and the American Cancer Society. Dr. Swanson was recently elected president of the American Society for Microbiology. She is co-host of the podcast This Week in Microbiology and co-author of the American Society of Microbiology Press textbook Microbe. She received a B.S. in biology from Yale University, her M.S. in genetics from Columbia University, and her Ph.D. in genetics from Harvard University.

PAUL W. J. J. van der WIELEN is a principle scientist at KWR Water Research Institute and guest researcher at the Laboratory of Microbiology at the Wageningen University. As head of the biological activity research group at KWR, he focuses on biological stability of drinking water, growth of opportunistic pathogenic microorganisms in water, (micro)biological processes in drinking water treatment, and microbial ecology in drinking water. He uses the latest state-of-the-art methods such as next generation sequencing to resolve microbial interactions in man-made water systems and to study the effect of measures to control microbial processes in these systems. Before working in the field of drinking water microbiology, he investigated the microbial ecology of the gastrointestinal tract, deep hypersaline lakes, and marine sediment. His work on *Legionella* at KWR focuses on the influence of water quality, pipe materials, and taps on growth of *Legionella pneumophila* and method development to detect *L. pneumophila*. He is co-editor of Microbial Growth in Drinking-Water Supplies published by International Water Association Publishing in 2014. Dr. van der Wielen holds an M.Sc. in microbial ecology from the University of Groningen and a Ph.D. in microbial ecology from Utrecht University.

LAN CHI NGUYEN WEEKES is the director of physical resources at La Cité in Ottawa, Ontario, Canada. She was previously the senior mechanical engineer and one of the founders of InAIR Environmental Ltd., where she was involved in evaluating *Legionella* risk and creating management plans for building water systems in Canada, as well as addressing other indoor environmental quality issues such as thermal comfort, biological contaminants, and drinking water quality. Ms. Weekes has presented on the topic of *Legionella* in building water systems at the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) and the Indoor Air Quality Association (IAQA) conferences, as well as publishing articles in ASHRAE and the Canadian Consulting Engineer journal. She is currently helping to revise sections of the Canada Building Code to address potential *Legionella* issues in HVAC systems. Ms. Weekes is an author of the HVAC inspection section of the American Industrial Hygiene Association's Recognition, Evaluation, and Control of Indoor Mould book. She holds a B.M.E. from Polytechnique of Montreal and an M.A.Sc. in building environment from Concordia University.

Staff

LAURA J. EHLERS is a senior staff officer for the Water Science and Technology Board of the National Academies of Sciences, Engineering, and Medicine. Since joining the National Academies in 1997, she has served as the study director for more than 20 committees, including the Committee to Review the New York City Watershed Management Strategy, the Committee on Bioavailability of Contaminants in Soils and Sediment, the Committee on Assessment of Water Resources Research, the Committee on Reducing Stormwater Discharge Contributions to Water Pollution, and the Committee to Review EPA's Economic Analysis of Final Water Quality Standards for Nutrients for Lakes and Flowing Waters in Florida. Dr. Ehlers has periodically consulted for EPA's Office of Research Development regarding its water quality research programs. She received her B.S. from the California Institute of Technology, majoring in biology and engineering and applied science. She earned both an M.S.E. and a Ph.D. in environmental engineering at the Johns Hopkins University.