Legionella: a reemerging pathogen

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Purpose of review
The present review summarizes new knowledge about Legionella epidemiology, clinical characteristics, community-associated and hospital-based outbreaks, molecular typing and molecular epidemiology, prevention, and detection in environmental and clinical specimens.

Recent findings
The incidence of Legionnaire's disease is rising and the mortality rate remains high, particularly for immunocompromised patients. Extracorporeal membrane oxygenation may help support patients with severe respiratory failure. Fluoroquinolones and macrolides appear to be equally efficacious for treating Legionnaires' disease. Whole genome sequencing is an important tool for determining the source for *Legionella* infections and for understanding routes of transmission and mechanisms by which new pathogenic clones emerge. Real-time quantitative polymerase chain reaction testing of respiratory specimens may improve our ability to diagnose Legionnaire's disease. The frequency of viable but nonculturable organisms is quite high in some water systems but their role in causing clinical disease has not been defined.

Summary
Legionellosis remains an important public health threat. To prevent these infections, staff of municipalities and large buildings must implement effective water system management programs that reduce *Legionella* growth and transmission and all Medicare-certified healthcare facilities must have water management policies. In addition, we need better methods for detecting *Legionella* in water systems and in clinical specimens to improve prevention strategies and clinical diagnosis.

Keywords
amoebae, *Legionella pneumophila*, Legionnaires' disease, outbreak, surveillance, viable but nonculturable organisms

INTRODUCTION

The present review discusses results of recent studies on *Legionella* epidemiology, clinical characteristics, community-associated and hospital-based outbreaks, molecular typing and molecular epidemiology, prevention, and detection in environmental and clinical specimens. During the period covered by the review other review articles discussed specific aspects of *Legionella* in depth [1,2,3▪,4▪▪,5▪▪,6–9].

EPIDEMIOLOGY

Legionella pneumophila and other *Legionella* species are ubiquitous in natural aquatic environments and frequently contaminate man-made water systems [10,11▪,12,13]. Recent reports indicate that *L. pneumophila* is a significant public health issue [11▪,12–14,15▪,16,17▪,18▪,19,20]. Approximately 8000 to 18 000 patients are hospitalized with Legionnaires' disease in the United States each year [19], with an average length of stay of 10.2 days and an average cost of care of $26 741 to $38 363 [21].

The Centers for Disease Control and Prevention (CDC) collects data on *Legionella* infections through the National Notifiable Diseases Surveillance System (NNDSS), and Supplemental Legionnaires Disease Surveillance System (SLDSS), the Waterborne Disease and Outbreak Surveillance System (WBDOSS) [15▪,16], and the Active Bacterial Core Surveillance (ABCs) [14]. Data from these surveillance and reporting systems indicate that the incidence of

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KEY POINTS

- The incidence of *Legionella* infection has been increasing in the United States and in Europe.
- Because high-risk patients may be susceptible to low concentrations of *L. pneumophila* in water systems, healthcare facilities must consider any level of *Legionella* contamination at any site to be a hazard.
- The Centers for Disease Control and Prevention developed a toolkit to help facilities address the Centers for Medicare and Medicaid mandate that all Medicare-certified healthcare facilities have water management policies to reduce growth and transmission of *Legionella*.
- Whole genome sequencing is an important tool for assessing the epidemiology of *Legionella* within healthcare facilities and the community.

legionellosis has been increasing [1,14,16], with a nearly 3.5-fold increase between 2000 and 2011 in the United States [14]. In 2015, 6079 cases of legionellosis were reported to the NNDSS [22]. Legionellosis incidence varies by location, with rates at the 10 ABCs sites ranging from 0.4/100 000 in California to 4.0/100 000 in New York [14]. The three ABCs sites with the highest incidence (New York, Maryland, and Connecticut) are in the US Northeast or Mid-Atlantic regions. Legionellosis incidence is higher among blacks (1.5/100 000) than among whites (1.0/100 000). The incidence per 100 000 population also increases with age: 0.4 (<50 years), 2.5 (50–64 years), 3.6 (65–79 years), and 4.7 (≥80 years) [14].

Legionella accounted for 57–66% of drinking water-associated outbreaks reported to the WBDOSS during 2011–2014 and 13–26% of the cases [15▪,16]. More than half (57.1 and 76.8%) of the legionellosis outbreaks occurred between April and October. New York ($n=11$), Pennsylvania ($n=8$), Maryland ($n=6$), Florida ($n=6$), Ohio ($n=6$), and North Carolina ($n=5$) were the states that identified the most outbreaks. Of the patients who acquired legionellosis, 82.0–83.8% were hospitalized and 10–12.6% died [15▪,16].

Under the coordination of the European Centre for Disease Prevention and Control (ECDC), the European Legionnaires' disease Surveillance Network (ELDSNet) conducts surveillance for Legionnaires' disease in 29 European countries. ELDSNet reported that the age-standardized rate of all cases increased from 0.97 cases/100 000 population in 2011 to 1.30 cases/100 000 population in 2015, corresponding to an annual average increase of 0.09 cases/100 000 population (95% CI, 0.02–0.14; $P=0.02$) [23]. ELDSNet also reported several small clusters (two to three cases) of travel-associated cases that might reflect both a higher probability of clustering in a travel setting and near-real-time surveillance of travel-associated cases within ELDSNet [24].

During 2000–2014, CDC investigated 27 Legionnaires' disease outbreaks, which affected 415 people, 65 of whom died (15.7% overall; median outbreak case fatality rate 7%). The median number of cases was higher for cooling tower outbreaks ($n=22$) than for potable water outbreaks ($n=10$). Healthcare-associated outbreaks accounted for 57% of the cases and for 85% of deaths [25▪▪].

When CDC investigators reviewed data from 23 of the 27 outbreaks, they identified at least one water system maintenance deficiency for each outbreak [25▪▪]. Process failures were the most frequent deficiencies (15, 65%), followed by human errors (12, 52%), equipment failures (8, 35%), and unmanaged external changes (8, 35%). Specific deficiencies included inadequate water disinfectant levels (16 [70%]) and water temperatures in the optimal range for *Legionella* growth (12 [52%]). Nearby construction (3, 43%) and problems with water mains (3, 43%) were the most common unmanaged external changes. Most hot tubs and decorative fountains associated with outbreaks were not maintained adequately. Three affected buildings had water management programs, all developed before ASHRAE's standard was published in 2015 (see Prevention Section) [25▪▪].

CLINICAL CHARACTERISTICS OF LEGIONNAIRES' DISEASE

Two recent longitudinal studies provide new insights into clinical characteristics of Legionnaires' disease. Sivagnanam *et al.* [26] conducted a 15-year retrospective case review of *Legionella* infections. Of 32 patients, 22 (68.8%) were transplant patients. Twenty-one (95.5%) patients had a history of graft-versus-host disease, six of whom were on immunosuppressants for organ rejection. Eleven (50%) cases were caused by *L. pneumophila* and seven (31.8%) by *L. micdadei*. Two (9.1%) patients acquired their infections within 1 month of their transplants. One transplant patient had *L. pneumophila* bacteremia and sepsis and another had a rapidly progressive, fatal *L. micdadei* cellulitis. Ten (45.5%) transplant patients had severe complications, four (18.2%) required intubation, and three (13.6%) additional patients died of progressive respiratory failure. Seven (31.8%) transplant patients died (6/12 hematopoietic cell vs. 1/10 solid organ).

The authors noted several important points. First, the urine antigen test would have missed

59% of the cases. Second, *Legionella* infection can have unusual presentations in transplant patients [26]. Third, the mortality rate was high, despite appropriate treatment; all fatalities were caused by either *L. pneumophila* or *L. micdadei*. Thus, hospitals caring for transplant patients should know the epidemiology of *Legionella* infections in their facilities and their communities and should be able to do *Legionella* cultures so they can detect species other than *L. pneumophila*. Clinicians practicing in facilities or communities that have identified *Legionella* infections should consider this organism in transplant patients, even those who present with extrapulmonary symptoms.

Durando *et al.* described a patient with a fatal pneumonia whose blood cultures grew *L. pneumophila*. Water from the patient's room was positive by real-time polymerase chain reaction (RT-PCR) for *Legionella* and cultures grew 20 colony forming units (CFU)/l, indicating very low *L. pneumophila* concentrations pose a risk to susceptible hosts [27].

Kao *et al.* reviewed 32 cases of community-acquired Legionnaires' disease in Taiwan [28]. Fourteen (43.8%) patients had gastrointestinal symptoms, 10 (31.2%) had musculoskeletal symptoms, and 2 (6.2%) had neurological abnormalities. Eleven of 16 (68.8%) patients who did not receive appropriate treatment were admitted to an ICU compared with 5 of 16 (31.3%) who did. Seven (21.9%) patients died while hospitalized. These findings suggest that patients who present with signs and symptoms of a respiratory tract infection and extrapulmonary symptoms may have Legionnaires' disease. Early initiation of antibiotic treatment effective against *Legionella* may decrease the need for ICU care [28].

A recent case report suggests that under the right circumstances, *L. pneumophilia* can be transmitted from person to person [29▪]. The index case in an outbreak of Legionnaires' disease [30] performed maintenance on industrial cooling towers that were subsequently implicated. His mother, who was not exposed to the cooling towers, cared for him for 8 hours in a small nonventilated room when he was coughing intensely [29▪], subsequently acquired Legionnaires' disease. Both patients died and both were infected with the unique outbreak strain–*L. pneumophila* serogroup 1, ST1905 [29▪].

Miyashita *et al.* found that several clinical scores did not discriminate between patients with *Legionella* pneumonia and those with either *Streptococcus pneumoniae* pneumonia or *Mycoplasma pneumoniae* [31]. However, only 6% of the patients with *Legionella* pneumonia had fewer than two of the six clinical and laboratory parameters identified by Fiumefreddo *et al.* [32] compared with 21% for *S. pneumoniae* and 34% for *M. pneumoniae* ($P < 0.001$) [31]. Miyashita *et al.* also found efficacy rates of 94.5% for intravenous treatment (ciprofloxacin 94.5% [49/52] and pazufloxacin 94.2% [39/41]) and 95.5% for oral antibiotics (ciprofloxacin 100% [6/6], levofloxacin 91.7% [11/12], garenoxacin 100% [36/36], moxifloxacin 100% [2/2], clarithromycin 9/11 [81.8%]) [31].

After propensity matching, Gershengorn *et al.* found mortality rates of 6.3% [95% confidence interval (CI), 4.6–7.9%] for fluoroquinolones and 6.5% (95% CI, 4.8–8.2%; $P = .84$) for azithromycin [33]. Mortality rates for severely ill patients, hospital length of stay, incidence of *Clostridium difficile* infection, and total hospital cost did not differ between treatment [33]. Garcia-Vidal *et al.* [34] found that time to defervescence, time to clinical stability, length of intravenous therapy, length of hospital stay, and mortality rates did not differ significantly between patients with community-acquired *Legionella* pneumonia treated with levofloxacin and those treated with azithromycin. Patients treated with clarithromycin had longer durations of intravenous therapy and hospitalization than those treated with levofloxacin.

Of 112 patients rescued with extracorporeal membrane oxygenation (ECMO), the 14 patients with *Legionella* pneumonia had significantly higher respiratory system static compliance, required significantly higher ECMO support, had a significantly shorter duration of mechanical ventilation, and a significantly lower incidence of ICU-acquired healthcare-associated infections [35] than other patients. The survival rate was higher for patients with *Legionella* infections but the difference was not significant (85.7 vs. 62.2%; $P = 0.13$). The investigators concluded that patients with *Legionella* pneumonia complicated by refractory respiratory failure benefited from ECMO support.

OUTBREAKS

Much of our knowledge about epidemiological and clinical aspects of *Legionella* infections has come from outbreak investigations [2]. However, only 4% of cases reported to CDC are outbreak associated [25▪▪]. *L. pneumophila* serogroup 1 strains that react to monoclonal antibody 2 (MAb2) have caused more than 94% of outbreaks investigated by CDC [36].

Community-based outbreaks and transmission

Recently, several community-based and hospital outbreaks have garnered considerable attention [11▪,12,13,18▪,37]. Genesee County, Michigan,

experienced two large clusters of Legionnaires' disease in 2014 and 2015. Of the 88 affected persons, 12 died [12]. In April 2014, Flint stopped purchasing water from the Detroit Water and Sewer Department (DWSD) and began treating water from the Flint River. Seventy percentage of cases had known exposure to Flint drinking water. After Flint re-contracted with DWSD and implemented enhanced corrosion control, the number of Legionnaires' disease cases decreased to approximately that reported in 2010–2013 [12]. Direct evidence that Flint water was the outbreak source was not available. However, studies assessing Flint water during the outbreak period found conditions that facilitate *Legionella* growth, including 8.6 times increase in water corrosiveness, warm water temperature, and 1.3–2.2 times increase in water main breaks [11▪,12]. Furthermore, the number of *Legionella* spp. and *L. pneumophila* gene markers, which were higher than previously reported for US tap water [12], decreased after the city switched back to Detroit water [11▪]. Schwake *et al.* concluded that distribution system water chemistry and premise plumbing conditions could synergistically stimulate *Legionella* amplification [12]. They recommended that water utilities and other entities involved in the design, regulation, and management of premise plumbing systems collaborate to avoid future outbreaks by developing water utility guidelines for minimizing risk of community-level outbreaks, creating communication channels with building operators regarding water quality, and coordinating *Legionella* monitoring [12].

During 2015, New York City experienced a large outbreak of Legionnaires' disease – 138 cases with 16 deaths – associated with a contaminated cooling tower [18▪]. The outbreak was detected when daily spatiotemporal cluster analysis found that the number of cases in two South Bronx neighborhoods exceeded historical means by 7.6 and 24.5 standard deviations. Of the 138 affected persons, 108 (78.3%) lived in the outbreak zone and 16 (11.6%) lived elsewhere in the Bronx. The attack rate for the census tract that included building A was 356 per 100 000 population. Water samples from 21 of 55 (38.2%) cooling towers were positive by RT-PCR for *L. pneumophila* serogroup 1 and 14 (25.5%) grew the organism. Whole-genome sequencing (WGS) and epidemiologic evidence implicated the cooling tower associated with building A as the outbreak source [18▪].

A large ($n = 334$) cooling tower-associated community outbreak occurred in Portugal, during 2014 [30]. Investigators postulated that meteorological phenomena – wind from the north-east at 2–3 m/s, high humidity (~80%), and high concentrations of small particulate matter in the air – contributed to the large scale of this outbreak [30].

Two reports highlighted the fact that newborns can acquire *Legionella* infection during water births [17▪,38]. Investigations of the three cases, one fatal, revealed infection prevention gaps: inadequate chemical treatment of the water, water temperatures that facilitated *Legionella* growth, and use of a jetted nondisposable tub. The investigations revealed gaps in midwives' infection control practices during home water births [17▪,38]. The Arizona Department of Health Services developed educational resources and guidelines [39].

Healthcare-associated outbreaks

The Veterans Affairs Pittsburgh Healthcare System (VAPHS) experienced a prolonged Legionnaires' disease outbreak [13]. Six of 22 (27.3%) patients died. Investigators found that chlorine residuals in the potable water were 0.0–0.1 parts per million (ppm) and the water temperature ranged from 37.6 to 55.3° C. Twenty-three of 25 (92%) water samples grew *Legionella* but 22 had less than 10 ml. Three *L. pneumophila* serogroup 1 clinical isolates were identical by monoclonal antibody typing and sequence-based typing (SBT) to 11 environmental isolates. Mean copper–silver ion concentrations were at or above the manufacturer's recommended levels for *Legionella* control [13].

The investigation identified several issues [13]. First, the hospital based their additional remediation interventions on an action threshold of 30% of distal sites positive despite having cases at much lower positivity rates. Second, the hospital's protocol involved culturing swabs of biofilm and occasionally testing 100-mL water samples, which likely led to significant underdetection of *Legionella* in the water system given the low *Legionella* concentrations. Third, copper–silver likely was not effective given the very low chlorine residuals. Fourth, hospital staff likely did not recognize the problem promptly because they did not use CDC's definition of healthcare-associated Legionnaires' disease [13].

MOLECULAR TYPING TO CLARIFY THE EPIDEMIOLOGY OF *LEGIONELLA* INFECTIONS

DNA SBT is the current reference standard for typing *L. pneumophila* isolates [40]. Because a small subset of the more than 2000 sequence types account for a disproportionate percentage of clinical cases [10], SBT may not discriminate between outbreak-related and nonoutbreak related *Legionella* isolates and it cannot assess changes over time within an ST. Thus, WGS has become an important tool for clarifying

the epidemiology of these organisms [10,18▪,19, 40,41,42▪▪,43,44▪▪,45–48].

Recent investigations using WGS have found that *Legionella* infections in individual patients and among patients infected during an outbreak [45–48] were caused by more than one strain, which can confound cluster identification using standard phylogenomic methods [44▪▪]. Buultjen *et al.* demonstrated that a statistical learning approach based on *L. pneumophila* core genome single nucleotide polymorphisms (SNP) comparisons could define outbreak clusters and predict the source [44▪▪]. The model's assignments and the epidemiological data agreed for 93% of the isolates. The model's predictive ability was 86% for *Legionella* isolates from a hospital in another country [44▪▪].

WGS has been used in real time to identify the source of *Legionella* for individual patients [41] and for outbreaks [18▪,19,29▪,49] and to study the epidemiology of *Legionella* over time within a facility. For example, Bartley *et al.* found evidence for 'geographic microevolution' of *L. pneumophila* serogroup 1 within their hospital [19]. The isolates formed a single clade comprising three closely related subclades that diverged by a maximum of 31–63 SNPs. The investigators also identified subclade-specific plasmid types and mobile genetic elements that discriminated further between isolates [19].

Raphael *et al.* did WGS on *L. pneumophila* serogroup 1 isolates from 10 outbreaks in New York [50]. They found that the isolates from most outbreaks differed by less than five core SNPs and formed outbreak clades, however, isolates from one outbreak differed by 6–418 core SNPs. SNP analyses distinguished between isolates with indistinguishable PFGE profiles obtained from different outbreaks and also between closely related isolates collected from one hospital over 3 years.

WGS has shown that *L. pneumophila* is an ancient genetically diverse species [10] but its core genome is conserved across space and time [10,19,43,44▪▪]. Epidemiologically unrelated isolates obtained years apart can differ by as few as two SNPs, suggesting that *L. pneumophila*'s evolutionary rate related to point mutations is very low (<1 SNP/genome/year) [10,43]. In contrast, recombination plays a major role in *L. pneumophila* evolution [40,43] and likely is responsible for the emergence of new virulent strains such as ST47, the leading cause of legionellosis in north-western Europe [40]. David *et al.* [42▪▪] did WGS on *L. pneumophila* ST1 isolates from 17 hospitals. They found evidence for substantial diversity and ward-specific microevolution within the population [42▪▪]. In contrast, many epidemiologically unrelated isolates from the same region or from different countries varied by as few as 14 SNPs. They concluded that a low number of SNPs supports, but is not absolute evidence of, a link between isolates. WGS results indicating that a clinical isolate is nested within a clade is stronger genomic evidence of a link but such evidence can be obtained only if at least three water isolates from a facility are sequenced [42▪▪].

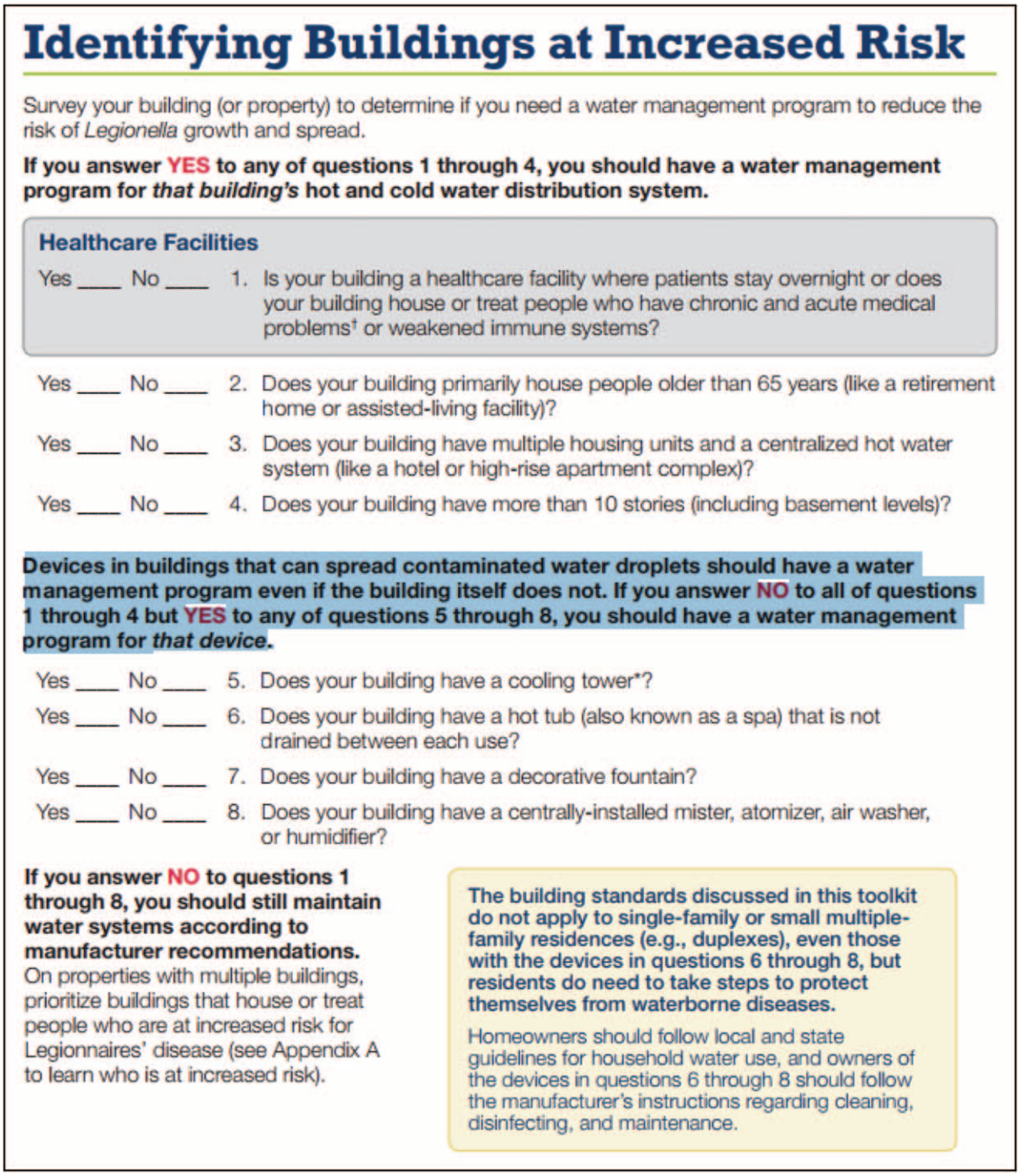
Subsequently, David *et al.* did WGS on 337 isolates from five sequence types that cause nearly half of the epidemiologically unrelated Legionnaires' disease cases in northwest Europe [10]. The genomic and phylogenetic analyses suggested that these sequence types emerged during the 20th century from different genomic backgrounds. Because the results were surprising for an environmental bacterium that 'accidentally' infects humans, the investigators suggested that: these *L. pneumophila* clones had adapted to new niches, likely associated with man-made water systems; the new niches drove the establishment and expansion of these strains; and clones adapted to specific niches are more likely than others to cause disease. If their hypothesis is correct, we must identify these environmental niches and the mechanisms by which these clones spread so that we can prevent infections.

PREVENTION

Two modeling studies have implications for *Legionella* control in plumbing systems [51,52]. Proctor *et al.* [51] found that temperature was more important than both pipe composition and the concentration of assimilable organic carbon for controlling *L. pneumophila* growth and that high temperatures decreased the effect of copper pipe. Cervero-Aragó *et al.* found that amoebae-associated *Legionella* decreased *Legionella* inactivation by chlorine and high temperature. They concluded that water close to the tap poses an increased health risk given the lower chlorine levels and temperatures [52].

To quickly decontaminate a hospital water supply, Bartley *et al.* flushed the system with a chlorinated alkaline detergent (pH = 10) to dissolve biofilm, superchlorinated the water to reach a residual of 10 mg/l free chlorine, and repeated this process three times until the water was macroscopically clear and without 'microbial contamination' [19]. They installed an in-line chlorinator on the water mains to achieve free chlorine levels of 1–4 mg/l at the point of use and they 'actively sought and removed' dead legs. Surveillance water cultures were negative for at least 16 months.

In 2015, ASHRAE published a consensus standard for primary prevention of Legionnaires' disease that requires facilities with large or complex building water systems to have a water management



Identifying Buildings at Increased Risk

Survey your building (or property) to determine if you need a water management program to reduce the risk of *Legionella* growth and spread.

If you answer YES to any of questions 1 through 4, you should have a water management program for *that building's* hot and cold water distribution system.

Healthcare Facilities

Yes ____ No ____ 1. Is your building a healthcare facility where patients stay overnight or does your building house or treat people who have chronic and acute medical problems† or weakened immune systems?

Yes ____ No ____ 2. Does your building primarily house people older than 65 years (like a retirement home or assisted-living facility)?

Yes ____ No ____ 3. Does your building have multiple housing units and a centralized hot water system (like a hotel or high-rise apartment complex)?

Yes ____ No ____ 4. Does your building have more than 10 stories (including basement levels)?

Devices in buildings that can spread contaminated water droplets should have a water management program even if the building itself does not. If you answer NO to all of questions 1 through 4 but YES to any of questions 5 through 8, you should have a water management program for *that device*.

Yes ____ No ____ 5. Does your building have a cooling tower*?

Yes ____ No ____ 6. Does your building have a hot tub (also known as a spa) that is not drained between each use?

Yes ____ No ____ 7. Does your building have a decorative fountain?

Yes ____ No ____ 8. Does your building have a centrally-installed mister, atomizer, air washer, or humidifier?

If you answer NO to questions 1 through 8, you should still maintain water systems according to manufacturer recommendations. On properties with multiple buildings, prioritize buildings that house or treat people who are at increased risk for Legionnaires' disease (see Appendix A to learn who is at increased risk).

The building standards discussed in this toolkit do not apply to single-family or small multiple-family residences (e.g., duplexes), even those with the devices in questions 6 through 8, but residents do need to take steps to protect themselves from waterborne diseases.

Homeowners should follow local and state guidelines for household water use, and owners of the devices in questions 6 through 8 should follow the manufacturer's instructions regarding cleaning, disinfecting, and maintenance.

FIGURE 1. CDC recommendation – Eight – screening questions to determine if the building or specific devices need a water management program. Data from CDC's Legionella Water Management Program toolkit [54▪▪].

program, including a risk assessment [53▪▪]. If the system is at risk, the institution must form a specialized management team to identify hazardous conditions and develop and implement specific control measures. CDC and its partners released a toolkit in June 2016 to help *Legionella* management teams comply with this standard [54▪▪]. CDC recommends asking eight screening questions to determine if the building or specific devices need a water management program (Fig. 1). In June 2017, the Centers for Medicaid and Medicare Services (CMS) mandated that all Medicare-certified facilities have water management policies that reduce *Legionella* growth and transmission [55▪▪]. In April 2017, Danila *et al.* [56] surveyed Minnesota hospitals and found that only 51% of the respondents knew about the ASHRAE

standard, 27% had water management plans, and 21% regularly tested water for *Legionella*.

DETECTION-ENVIRONMENT

Investigators from VAPHS demonstrated that water cultures were significantly more sensitive than concurrently collected swab cultures for detecting *L. pneumophila* (90 vs. 30% overall) [57]. Collins *et al.* found that quantitative real-time PCR (qPCR) had high negative predictive values (97.4–100%) but low positive predictive values (0–50%) [58▪]. They concluded that qPRC could help investigators quickly identify possible sources and rule out others.

Jinadatha *et al.* evaluated the sample volume, concentration, and limit of detection (LOD) needed to validate *Legionella* control in a facility. When they included all LODs, the percentage positive samples increased from 35.4% for 100 ml samples to 64% for 1000 ml samples for *L. pneumophila* serogroup 2–14 ($P < 0.01$). However, with LODs set to 1 or 10 CFU/ml, the smaller volumes (100, 250, 500 ml) were as sensitive as 1000 ml. The investigators argued that using 100 ml samples and an LOD of 1 CFU/ml was adequate for validating *Legionella* control given the amount of shower water patients likely inhale [59].

Nucleic-acid based detection methods are more rapid and sensitive than culture for detecting *Legionella*. For example, qPCR detected *Legionella* spp. in 57% of ultrafiltered drinking water samples from six sites [60]. However, these methods also detect nucleic acids from dead or dying bacteria, organisms associated with amoeba and viable but nonculturable (VBNC) legionellae [1]. Marinelli found that 7 of 42 (17.0%) tap water samples with negative cultures became positive for VBNC 15 days to 9 months after they were obtained [61▪]. To date, the clinical significance of VBNC *Legionella* has not been determined.

CLINICAL DETECTION

Avni *et al.* [62▪] conducted a systematic literature review to compare the diagnostic accuracy of PCR alone and urinary antigen testing for detection of *Legionella* spp.. The sensitivity and specificity of the urine antigen were 77.0% (55.3%-90.0%) and 99.9% (99.9%-99.9%) and those for PCR were 93.1% (63.9–99.0%) and 99.1% (98.0–99.5%). PCR identified 18–30% more *Legionella* infections than did the urine antigen. The investigators concluded that PCR on respiratory specimens is a valid diagnostic tool [62▪].

Fluoroquinolones-resistant *Legionella* have recently been identified among patients treated with these agents [63]. However, neither the Clinical and Laboratory Standards Institute (CLSI) nor the European Committee on Antimicrobial Susceptibility Testing [EUCAST] have developed standardized fluoroquinolones susceptibility tests. Thus, the frequency of fluoroquinolones-resistant is not known. Hennebique *et al.* found that digital PCR (dPCR) detected mutated *gyrA* sequences in mixtures of fluoroquinolones-resistant and susceptible *L. pneumophila* strains at 1 : 1000 resistant/susceptible allele ratios compared with ratios of 1 : 1 for Sanger sequencing and 1 : 10 for qPCR. dPCRgyrA detected small amounts of *gyrA* mutants in four samples (10.5%) from three (13.0%) patients [64]. These investigators suggested that dPCR could be used to detect patients at risk of treatment failure related to fluoroquinolones resistance [64].

CONCLUSION

The incidence of Legionnaire's disease continues to rise and the mortality rate remains high, particularly for immunocompromised patients. ECMO may help support patients with severe respiratory failure. WGS has become an important tool for determining the source for *Legionella* infections and for understanding routes of transmission and mechanisms of emergence for new pathogenic clones. We need better methods for detecting *Legionella* in water systems and in clinical specimens to improve prevention strategies and clinical diagnosis. Appropriate water system management in municipalities and in premises is essential for preventing these infections.

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Conflicts of interest

There are no conflicts of interest.

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