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Rapid Identification of a Cooling Tower-Associated Legionnaires' Disease Outbreak Supported by Polymerase Chain Reaction Testing of Environmental Samples, New York City, 2014–2015

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Abstract

We investigated an outbreak of eight Legionnaires' disease cases among persons living in an urban residential community of 60,000 people. Possible environmental sources included two active cooling towers (air-conditioning units for large buildings) <1 km from patient residences, a market misting system, a community-wide water system used for heating and cooling, and potable water. To support a timely public health response, we used real-time polymerase chain reaction (PCR) to identify *Legionella* DNA in environmental samples within hours of specimen collection. We detected *L. pneumophila* serogroup 1 DNA only at a power plant cooling tower, supporting the decision to order remediation before culture results were available. An isolate from a power plant cooling tower sample was indistinguishable from a patient isolate by pulsed-field gel electrophoresis, suggesting the cooling tower was the outbreak source. PCR results were available <1 day after sample collection, and culture results were available as early as 5 days after plating. PCR is a valuable tool for identifying *Legionella* DNA in environmental samples in outbreak settings.

Introduction

Legionnaires' disease (LD) is a severe pneumonia that can be accompanied by gastrointestinal and neurologic symptoms. Risk factors include smoking, age >50 years, and comorbidities. Onset occurs 2–10 days after exposure to *Legionella*, a genus of intracellular gram-negative bacteria found in water and soil. *L. pneumophila* serogroup 1 (LP1) causes 65–90% of cases for which there is a bacterial isolate (Bennett, Dolin, & Blaser, 2014). The majority of cases are diagnosed by urine antigen test (UAT), a rapid test that is highly sensitive and highly specific for LP1 and is widely available in acute care settings. Identification of the environmental source relies on comparison of patient isolates and environmental isolates by molecular techniques. Culture of respiratory specimens is necessary to obtain patient isolates, but LD patients might not produce sputum, specimens are not routinely cultured on *Legionella*-specific media, and specimens are less likely to yield positive culture results if they are collected after a patient has begun antibiotic therapy.

LD outbreaks have occurred from exposure to bioaerosols from cooling towers, decorative fountains, hot tubs, market misting systems, and potable water systems in hospitals, hotels, and residential buildings (Cunha, Burillo, & Bouza, 2016; Fraser et al., 1977; Haupt et al., 2012; Mahoney et al., 1992). Cooling towers have caused large community outbreaks including an outbreak with 334 cases in Portugal in 2014 and an outbreak with 449 confirmed cases in Spain in 2001, and likely have caused many sporadic cases (Bhopal & Fallon, 1991; García-Fulgueiras et al., 2003; Shivaji et al., 2014). Bioaerosols from cooling towers can travel substantial distances and have caused illness among persons up to 11.6 km away from a source (White et al., 2013). Recovery of a bacterial isolate by culture is the standard for identification of *Legionella* in the environment. PCR can detect *Legionella*

DNA, but does not indicate the presence of viable bacteria or provide a quantitative measure of the degree of contamination.

An estimated 8,000–18,000 cases of LD requiring hospitalization occur annually in the U.S. (Marston et al., 1997). Approximately 200–300 cases are reported annually in New York City; the age-adjusted incidence rate rose from 0.6/100,000 population during 2000 to 2.5/100,000 population during 2014, peaking at 3.4/100,000 population during 2013 (New York City Department of Health and Mental Hygiene [DOHMH], 2015a). This rise might be due to increased use of UAT by healthcare providers, an increase in the population at risk, or changes in the number and maintenance of cooling towers and their colonization by *Legionella* (Farnham, Alleyne, Cimini, & Balter, 2014). During 2000–2013, Bronx County had between 7–72 cases/year (crude rate 0.5–5.2 cases/100,000 population/year).

Clinical laboratories in New York City report positive *Legionella* test results to the New York City Department of Health and Mental Hygiene (DOHMH). For each case, DOHMH personnel review medical records to confirm illness and interview the patient or a close relative to determine possible *Legionella* exposure sources at home, work, healthcare settings, or associated with travel. Identification of a cluster of cases in space and time without a common building exposure indicates an outdoor exposure to a cooling tower or other outdoor aerosol source. An epidemiologist reviews all cases for common exposures and we also detect clusters at the city-, county-, and neighborhood- (multiple ZIP code) levels with a weekly automated system that compares the number of cases diagnosed in the past 4 weeks with that period and the prior and following 4-week periods in the previous 5 years, a modified historical limits method (Levin-Rector, Wilson, Fine, & Greene, 2015).

In December 2014 we identified a cluster of LD cases through a combination of epidemiologist review and automated cluster detection. All cases were located in Co-op City, a 1.3-square-kilometer residential neighborhood in northeastern Bronx County that is home to 60,000 persons, many retired, living in 15,372 residential units, including 14,900 apartments in 35 high-rise towers (24–33 floors) and 472 townhouses in 7 groups. All of Co-op City is contained within ZIP code 10475.

On December 1, 2014, the automated system reported nine cases among persons living in Bronx County (a larger area surrounding Co-op City) over the prior 4 weeks. Review of the four completed interviews found no common building exposures but found that two patients resided in Co-op City. On December 22, the automated system reported 12 cases among persons living in Bronx County over the prior 4 weeks, including four cases in Co-op City. We investigated to determine the magnitude and source, and to prevent further illness.

Methods

Case Surveillance

We defined an outbreak-associated case as LD diagnosed by UAT or culture and radiographic evidence of pneumonia in a person who lived in Co-op City with illness onset during November 2014–January 2015. Initial investigations found no common buildings visited by five patients.

On January 6, 2015, we alerted healthcare providers in New York City about the increase in cases in Bronx County and asked them to collect respiratory tract specimens to culture for *Legionella* from patients with respiratory symptoms, consider treating those patients for LD, and send isolates to the New York City Public Health Laboratory (PHL) (DOHMH, 2015b). We asked hospital infection control staff to identify stored respiratory tract specimens from recent patients with respiratory symptoms and to culture these specimens for *Legionella*. DOHMH held a community meeting in Co-op City on January 13 and issued a press release to inform the community about this investigation.

Environmental Investigation

At the time, there was no definitive source of information on the locations of cooling towers in New York City. We identified possible environmental sources in Co-op City— including cooling towers, markets with misting systems, and decorative fountains—by using environmental assessments, patient interviews, review of satellite imagery, and field visits. We also identified cooling towers in city administrative data for building owners who had requested a financial credit for wastewater reduction.

We collected samples at all identified environmental sources. Water and swab samples were collected at multiple points in cooling towers and the other suspected sources; pH, chlorine, and temperature were also tested. At cooling towers, we sampled from the surface of the cooling tower water pool, which could reflect aerosol content, and from stagnant water and biofilm, which are thought most likely to harbor bacterial overgrowth. Water samples were stored in 500 mL sterile containers treated with sodium thiosulfate (0.5 mL of a 0.1 N solution). We reviewed cooling tower maintenance practices for any deficiencies.

Samples were split between PHL and the New York State Department of Health Wadsworth Center (WC). Health department security staff transported samples by car overnight to WC for next-day testing. Environmental samples were screened for *Legionella* DNA at WC by a previously described real-time PCR assay to detect and differenti-ate *Legionella* species, *L. pneumophila*, and *L. pneumophila* serogroups 1–16; the assay includes an internal control for inhibitory substances. Validation testing suggested that a negative PCR result does not need to be confirmed with bacterial culture (Mérault et al., 2011; Nazarian, Bopp, Saylors, Limberger, & Musser, 2008).

Samples in which *Legionella* DNA was detected were processed and cultured starting on the same day, at WC and PHL, to obtain *Legionella* isolates. Environmental isolates and patient isolates were serogrouped by direct fluorescent antibody testing and typed by pulsed-field gel electrophoresis (PFGE) by Sfi1 digest (Sabrià et al., 2001). PFGE patterns of patient isolates and environmental LP1 isolates were compared using BioNumerics software.

Results

Case Surveillance

We identified eight cases in Co-op City through routine surveillance. Illness onset ranged from November 4–December 28, 2014, and diagnosis ranged from November 9, 2014–January 6, 2015. New cases were reported to the health department in every week of

December; the last case was reported on January 8. All patients were male. Seven (88%) were smokers. Four (50%) had underlying comorbidities including diabetes, cardiovascular disease, chronic hepatitis C, chronic kidney disease, asthma, hypertension, and HIV. The mean age was 57.5 years (range 29–69 years). All were hospitalized, and none died. Sputum from one patient grew *Legionella* and an isolate was sent to PHL. Two (25%) lived in the same building.

Surveillance is ongoing in New York City and no additional cases have been subsequently reported in residents of Co-op City in the following 6 months after the last case described here.

Environmental Investigation

We located two active cooling towers in Co-op City, both within 1 km of all case patient homes. One cooling tower was located at a 40-megawatt power plant and had five cells, including two that ran year round and three that ran during April–October each year. A second cooling tower was located on top of a shopping mall and had one cell that ran year round. We also identified an inactive cooling tower.

Environmental assessment of the residential complexes revealed a community-wide closed-loop water system that ran to all Co-op City housing units and passed hot or cold water over coils for heating or cooling, resupplied by municipal water. One market had a vegetable misting system, but no patients had visited that market. We sampled all of these sites, apartments where two patients lived, and the municipal water supply. We found no decorative fountains or whirlpool spas in Co-op City.

PCR screening detected LP1 at the power plant cooling tower and *L. pneumophila* serogroup 6 (LP6) at the power plant cooling tower and at the mall cooling tower. The community-wide closed-loop water system, market misting system, residential housing, and municipal water had no detectable *Legionella* DNA, with all results received <1 day after specimen collection (Table 1).

After PCR detected LP1 at the power plant cooling tower, which was also the only known source with a wide aerosol distribution that could account for all cases, we proceeded to require its shutdown and remediation. On January 9, the same day LP1 DNA was detected by PCR, a health commissioner order was sent to power plant staff and the cooling tower was shut down; remediation began January 12 after development of a remediation plan. After three rounds of shock disinfection, no further LP1 grew in samples collected on January 26. The power plant cooling tower resumed operation on January 26 during extreme cold weather, as the plant power was needed to provide power and heat to the community.

Culture, isolation, and typing were completed later: LP1 grew in samples from the power plant cooling tower and LP6 grew in samples from the power plant cooling tower and the mall cooling tower, with results received on January 14, 5 days after PCR results. Samples from other locations were not placed in culture after testing negative by PCR. LP1 from the power plant cooling tower was indistinguishable from the patient isolate by PFGE (Figure 1, lanes 3–5).

Legionella testing at the power plant cooling tower occurred once per year in the summer, most recently in July 2014, with no *Legionella* detected in 7 years of operation. Review of maintenance records from August–December 2014 revealed that staff had changed the disinfection biocide in August 2014 from a bromine-based pellet to a chlorine-based liquid, added over a 1-hr period daily with a target total chlorine level of 2–4 ppm. Samples collected 2 hr after the biocide was added were tested for total chlorine; however, the target range was not adjusted for the new biocide formulation and free chlorine was not routinely measured.

Following shutdown and remediation, no *Legionella* grew in follow-up samples collected from the power plant cooling tower every 2 weeks through March 18. Follow-up testing included monthly testing for *Legionella*; for counts >1,000 CFU/mL, remedial action was to be performed by dosing water with a chlorine-based compound equivalent to 5 mg/L of free residual chlorine for at least 1 hr, and then *Legionella* culture was to be performed every 3–7 days until two consecutive negative samples were obtained. DOHMH also recommended that the cooling tower at the mall be shut down and remediated out of an abundance of caution.

Discussion

We investigated an outbreak of eight Legionnaires' disease cases linked by environmental and laboratory data to an industrial cooling tower at a power plant. This investigation was notable for the use of PCR screening of environmental samples to implicate one cooling tower and support shutting it down for remediation, several days before culture results were available. The implicated cooling tower had a recent change in biocide formulation that might have allowed lower biocide levels and more favorable conditions for *Legionella* growth. Review of cooling tower maintenance plans found deficits; testing identified viable *Legionella* bacteria, both common findings in other cooling tower assessments in New York City and elsewhere (Mouchtouri, Goutziana, Kremastinou, & Hadjichristodoulou, 2010).

Industry guidelines illustrate best practices for testing and maintenance to prevent *Legionella* overgrowth; these interventions, however, might not remove *Legionella* (American Society of Heating, Refrigerating and Air-Conditioning Engineers, 2015; Cooling Technology Institute, 2008). Remediation at this cooling tower required shutdown for multiple days and implementation of a water safety plan with more monitoring and data collection to guide improvements in process control. We recommend that all cooling towers should have a written water safety plan and should test regularly to ensure adequate disinfection.

Multiple limitations to this investigation are noted. PCR detects DNA and does not indicate the presence of viable bacteria. Limited sampling from each environmental site might have led to a false negative result when other strains of *Legionella* were present. For patients, UAT primarily detects LP1 but has cross-reactivity for other serogroups; mixed infections are also possible. For seven (88%) cases, we did not have respiratory tract cultures or isolates, which means we do not have information on their strains or serogroups. We also might have missed cases of illness among persons who were not tested for *Legionella* infection or who did not live in Co-op City but were exposed there or nearby.

An increase in cases might reflect increased clinician awareness and testing practices following our health alert; however, only one case was diagnosed after that alert. Finally, during this investigation we were limited to testing those cooling towers that we were able to identify in administrative data or aerial imagery. In August 2015, after a large LD outbreak elsewhere in Bronx County caused by a cooling tower, the New York City Council enacted comprehensive rules for cooling tower registration, testing, and maintenance (City of New York, 2015). That registry identified four additional cooling towers in Co-op City that had not been identified through our initial source-finding methods, highlighting the need for registration systems or other investigative approaches to facilitate public health investigations.

Conclusion

While this outbreak progressed, we were identifying new cases weekly. We were concerned that deaths and more cases might occur before we could identify the source and take public health action. The power plant cooling tower was the most likely source of aerosol that could expose the entire community and we were prepared to require remediation there, but we wanted laboratory evidence of *Legionella* contamination before taking action. Waiting for culture results would have delayed action by several days.

We balanced the importance of rapid source control and remediation with the practical implications of shutting down, cleaning, and testing the power plant that was the sole power source for 60,000 persons, on short notice during a period of extreme cold weather. These actions were also expensive, as the cooling tower operators spent approximately $750,000/week for backup power during the shutdown period.

We were able to obtain PCR results <1 day after sample collection and the cooling tower was shut down 1 day later. Culture results, received as early as 5 days after plating, confirmed the PCR findings. The *Legionella* PCR assay used by WC has an analytic sensitivity of <1 CFU and a specificity of 100%; prior use found substantial agreement with culture (Nazarian et al., 2008). This PCR assay contains a target for inhibitory growth that makes it a valuable tool for screening before culture, because a negative result indicates culture is not needed. Negative PCR results at other possible sources strengthened the evidence for the power plant cooling tower as the source. Since this outbreak, we have incorporated PCR testing for environmental *Legionella* DNA into our protocol for LD investigations to more rapidly identify potential sources. We recommend that other jurisdictions also consider its use in outbreak settings.

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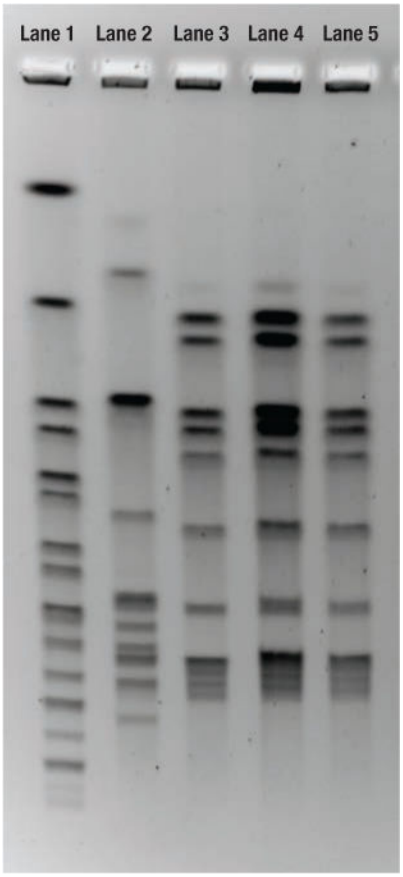

FIGURE 1. Legionella pneumophila

Pulsed-Field Gel Electrophoresis Patterns (Sfi1 Digestion)

From left to right: lane 1, molecular weight standard H9812; lane 2, historic case (clinical specimen); lane 3, outbreak case (clinical specimen); lane 4, power plant cooling tower (environmental sample 1); and lane 5, power plant cooling tower (environmental sample 2).

TABLE 1

Detection of *Legionella pneumophila* in Environmental Samples, New York City, 2014–2015

Sampling Location	PCR*	Culture*	Serogroup
Power plant cooling towers	29/30	27/30	LP1, LP6
Shopping mall cooling tower	8/10	1/8	LP6
Residential housing (two apartments)	0/23	–	–
Community-wide closed-loop water system	0/4	–	–
Vegetable misting system in market	0/5	–	–
Public potable water supply	0/2	–	–

PCR = polymerase chain reaction; LP1 = *L. pneumophila* serogroup 1; LP6 = *L. pneumophila* serogroup 6.

*Results are number of positive samples divided by total samples tested. Only specimens positive or inconclusive for *L. pneumophila* by PCR were cultured; negative specimens were not cultured.