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Clinical Microbiology
NEWSLETTER

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CMN
Vol. 37, No. 15
August 1, 2015
www.cmnewsletter.com

IN THIS ISSUE



# *Legionella*: a Fascinating Bacterium Uncovered in the Twentieth Century

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## Abstract

In July 1976, the American Legion held a conference at the Bellevue Stratford Hotel in Philadelphia, PA, to celebrate the nation's bicentennial. This convention resulted in transmission of a gram-negative bacterium to over 200 attendees, who developed a respiratory illness; 34 deaths were attributed to the infections. An investigation of the illness revealed a bacterium that had not been documented before. The disease became known as Legionnaires' disease, and the etiological agent was subsequently named *Legionella pneumophila*. This is the story of *Legionella*, with special emphasis on its ecological niche, the diagnosis of human infection, and its isolation from the environment.

There are only a handful of diseases that debuted in the 20th or 21st century. They include Legionnaires' disease (the subject of this review), Lyme disease, AIDS, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and West Nile virus.

## The Beginning

In late July 1976, there was an outbreak of severe pneumonia among individuals attending an American Legion convention at the Bellevue Stratford Hotel in Philadelphia, PA. Two hundred twenty-one individuals, predominantly men, were stricken, and 34 died. The investigation that followed identified a new bacterium, which was subsequently named *Legionella pneumophila* (1, 2). Almost 50 years later, Legionnaires' disease is a nationally notifiable disease in the United States (3). The CDC estimates that between 8,000 and 18,000 cases of Legionnaires' disease occur in the United States annually (4). In addition, between 1995 and 2005, over 32,000 cases were reported to the European Working Group for *Legionella* Infections (5). It has subsequently been learned that an unidentified gram-negative rod that was frozen in 1947 was later identified as *Legionella* sp., so the American Legion convention was not the first time *Legionella* was isolated from humans (6).

Hospital-acquired *Legionella* pneumonia has a fatality rate of 12% (7), with *L. pneumophila* causing 80% of human infections. The organism causes two different illnesses, the typical Legionnaires' disease, which is a severe form of pneumonia, and Pontiac fever, a flu-like illness with a much milder course and, usually, no fatalities. *Legionella* spp. are not transmitted person to person but through the air and in water. Symptoms of Legionnaires' disease include fever, a nonproductive cough, headache, myalgias, rigors, dyspnea, diarrhea, and delirium. Erythromycin and, later, ciprofloxacin were designated the initial drugs of choice for the treatment of legionellosis; however, newer macrolides, such as azithromycin or clarithromycin, and newer quinolones, such as levofloxacin, moxifloxacin, and gemifloxacin, have also been found to be effective (8).

## Diagnosis of Human Infection

The traditional gold standard for the diagnosis of *Legionella* infections is culture. However, legionellae will not grow on routine laboratory media, such as blood and chocolate agars; therefore, it is important for the clinician to notify the laboratory that *Legionella* is suspected so that the appro-

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priate media are set up. The organism grows best on buffered charcoal yeast extract (BCYE) agar; this medium contains cysteine and iron, which are required by this environmental organism.

Clinical microbiology laboratories that perform cultures for *Legionella* usually use BCYE, a non-selective medium, and BCYE with polymyxin B, anisomycin, and cefamandole, a selective medium designed to inhibit the growth of normal respiratory organisms, which may be present in higher density and compete with the *Legionella* spp. in sputum or other specimens, such as bronchial alveolar lavage fluids.

After 72 to 96 hours of incubation, colonies of *Legionella* are round and convex with entire edges (Fig. 1); the colony itself is described as having a ground-glass (or iridescent) appearance under the stereoscope. Most *Legionella* strains are oxidase variable and negative (inert, or non-reactive) for most standard biochemicals, such as nitrate reduction, urease, and carbohydrate utilization. Most, however, will liquefy gelatin and are beta-lactamase positive. Lack of activity in standard biochemicals essentially makes phenotypic identification impossible. Confirmation of identification is usually performed by staining with polyvalent conjugates containing monoclonal antibodies against the most common clinical isolates. Therefore, recognizing *Legionella* morphologically on BCYE and serogrouping the isolate is the most rapid and cost-effective way to diagnose it.

A urinary antigen test that detects *Legionella* antigens in a patient's urine is also available. Unfortunately, the test detects only *L. pneumophila* serogroup 1. In addition, *Legionella* urine antigen can remain positive for 3 months to 1 year after exposure (9). A direct fluorescent antibody test is available to stain lung tissue and also to confirm the genus identification of a clinical isolate from a culture, as described above. Finally, serological testing is available to detect a 4-fold rise in titer from acute to convalescent sera of a patient suspected of having Legionnaires' disease. A single antibody titer of ≥1:256 used to be indicative of Legionnaires' disease pneumonia. However, it no longer is, as many asymptomatic individuals have been found to have single high titers.

PCR is the current test of choice for laboratories that have self-validated laboratory-developed tests, as there are no FDA-cleared commercial assays (10).

### Risk Factors for Acquiring Legionnaire's Disease

Epidemiological investigations have shown that (i) increased age (>50 years), (ii) smoking, (iii) male sex, (iv) history of chronic lung disease, (v) hematologic malignancies, (vi) end-stage renal disease, (vii) lung cancer, (viii) immune suppression, and (ix) diabetes are predisposing factors for acquiring *Legionella* (11).

### Where Does *Legionella* Live?

Table 1 shows the long list of environmental sources that have been associated with *Legionella* infections. In recent years, novel sources of *Legionella* have appeared, such as windshield wiper fluid (12) and misters in a grocery store vegetable section (13). The one commonality to the ecology of the microbe is water. In fact, the natural habitat for *Legionella* is water.

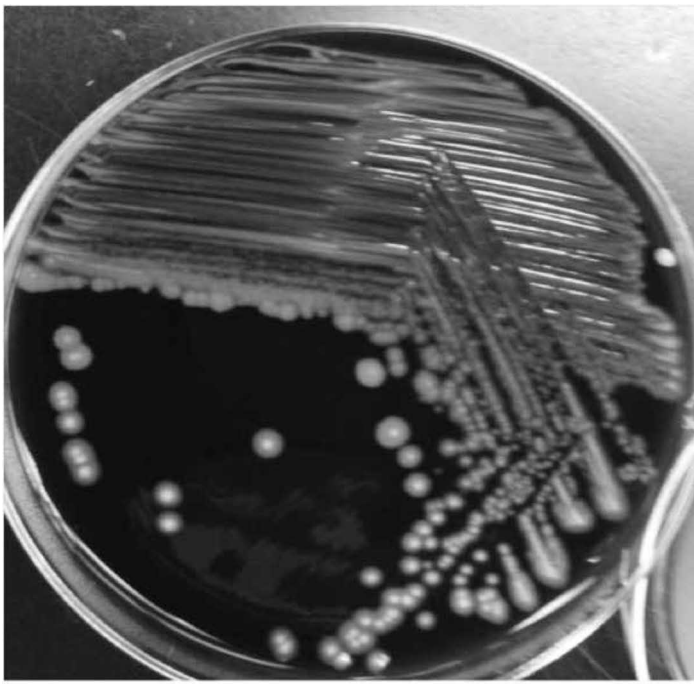

**Figure 1.** *L. pneumophila* on BCYE.

In the course of doing business, our environmental laboratory has cultured *Legionella* from many environmental sources. Health care facilities represent a disproportionate amount of the cultures we perform. The state of Texas, where we live and work, has published

**Table 1**. Aquatic sources of *Legionella*

| |
|---|
| Central air conditioning systems in office buildings, hotels, and hospitals |
| Cooling towers used in industrial cooling systems' evaporative coolers |
| Metal-working fluids used in industry as coolants and lubricants |
| Nebulizers[a] |
| Room air humidifiers[a] |
| Whirlpool spas or hot tubs |
| Water heating systems (especially electric hot water heaters in commercial buildings) |
| Showers (especially showers with a handheld wand or water saver system) |
| Ice-making machines |
| Decorative indoor fountains[b] |
| Windshield wiper fluid[c] |
| Misting systems in the produce section of grocery stores |
| Dental equipment |
| Hydrotherapy pools |
| Fire sprinkler systems |

[a] Distilled or sterile water should be used to fill nebulizers and humidifiers, as tap water may contain *Legionella*.
[b] Submerged lighting acts as a heat source.
[c] Studies showing *Legionella* in windshield wiper fluid were presented 18 May 2014 at the American Society for Microbiology General Meeting; the patients in 75% of cases occurring in one Arizona school district had *Legionella* in their windshield washer fluid.

a special task force report on Legionnaires' disease because of the number of human infections found in the state (14). Health care facilities are supposed to collect specimens for testing twice a year.

*Legionella* grows in water sources whose higher (warmer) temperatures allow the thermotolerant bacteria to thrive and multiply. These niches include hot water tanks and domestic hot water sources. Outdoors, in ponds, lakes, and other aquatic systems, *Legionella* lives with and within amoebae in a symbiotic relationship (15,16). It grows in water at temperatures from 20°C to 50°C (68°F to 122°F) and particularly likes temperatures between 35°C and 46°C (95°F to 115°F). *Legionella* can also grow in ambient, untreated waters. These water sources may be shallow wells or non-chlorinated water tanks used for watering animals or for irrigation.

As an intracellular parasite of free-living amoebae like *Acanthamoeba*, *Legionella* survives within biofilms in building water systems. The ability to multiply inside protozoa, which was first described by Rowbotham (17), mimics its actions in human infections, where pathogens like *L. pneumophila* enter the body by way of the respiratory tract, are engulfed by macrophages, and multiply inside them.

## Performing an Investigation to Determine the Source of *Legionella*

The Occupational Safety and Health Administration (OSHA) publishes guidelines for the investigation of a *Legionella* outbreak in a hospital (18); the steps, however, could equally apply to investigations in office buildings or hotels. Table 2 shows the steps in an investigation. In addition, the CDC has published an environmental protocol (19) addressing how to select appropriate sites to sample. Whenever possible, 1 liter of water should be collected. Samples should be collected in sterile screw-cap polypropylene plastic bottles. Sodium thiosulfate should be added to neutralize any residual or free chlorine from domestic water sources. Swabs of faucets (necks and aerators) and showers (wands, necks, and heads) should be collected prior to collecting the water samples; the shafts are then broken off, and the swab is submerged in the water taken from that site. Samples should be transported back to the laboratory (ambient) in insulated coolers as protection against temperature extremes. Samples that will not reach the laboratory within 72 hours or will not be processed within 72 hours should be kept under air conditioning or refrigerated to control overgrowth of other environmental organisms.

*Legionella* is not often sought in the air, but air samples, if needed, can be collected using BCYE agar plates in a volumetric air sampler or in a glass impinger using liquid BCYE medium. (This liquid medium is similar to that used to do *Legionella* blood cultures.)

## Performing Environmental *Legionella* Cultures

To recover *Legionella* from water, the water should be either centrifuged (down to 10X of its initial concentration) or filtered through a 0.22-µm filter prior to inoculation onto BCYE and BCYE with polymyxin B, anisomycin, and vancomycin or BCYE with glycine, vancomycin, polymyxin B, and cycloheximide. The (10X) pellet or filter (resuspended in 1/10 of the supernatant) is then heated (20) or acid treated (HCl-KCl) to rid the sample of other heterotrophic bacteria. The culture is then incubated for 7 days; *Legionella* usually grows in 4 or 5 days.

If no *Legionella* bacteria grow in a highly suspicious setting, co-culture heat enrichment can be attempted (20). This technique takes into account the fact that *Legionella* bacteria may be present at low numbers in certain water samples but will continue growing inside their protozoan hosts. The remaining unconcentrated sample should be incubated at 35°C and subcultured at 2-week intervals for as long as 6 weeks. This procedure is said to increase the ultimate recovery of *Legionella* from positive samples by as much as 30%.

## Remediating *Legionella* in a Hospital, Hotel, or Office Building

There are a number of methods to control (or inhibit, but not kill) the growth of *Legionella* in buildings (21). The first method is via temperature control. If all cold (chlorinated) water is kept at ≤25°C (≤78°F) and all hot water is kept at ≥51°C (≥124°F), there should not be any systemic problem with *Legionella*. However, in most commercial buildings, hot water is maintained at closer to 110°F because of high energy costs. Electric hot water heaters also do not heat as evenly as gas. The water temperature may be 15 to 20°F lower at the bottom of the tank than at the top of the heater. Superheating the water to >60°C (>140°F) can help dimin-

**Table 2.** OSHA guidelines for the investigation of a hospital facility[a]

| | |
|---|---|
| I. | Review of the plumbing system including: |
| | Hot and cold domestic water sources |
| | Water heaters |
| | Distribution pipes |
| | Water coolers |
| | Water treatment equipment |
| | Connections to process water systems |
| | Storage tanks |
| II. | Review of HVAC system |
| | Cooling towers |
| | Evaporative condensers |
| | Fluid coolers |
| | Humidifiers |
| | Direct and indirect evaporative air-cooling equipment |
| | Air washers for filtration |
| | Note locations of fresh air intakes of the HVAC units relative to water sources, such as cooling towers. |
| III. | Look for other potential sources |
| | Decorative fountains |
| | Whirlpools, spas, hydrotherapy baths |
| | Plant misters |
| | "Dead legs" (unused or low-lying sections of plumbing pipes (stagnant water can pool) |

[a] Adapted from reference 18.

ish the *Legionella* bioburden for short periods but must be repeated every 3 to 5 weeks. For disinfection, hospitals use 180°F water for a 2-hour flush. This is performed by zones so that patient care is not affected. Maintaining water temperatures too high (>140°F) has resulted in patient scalding incidents, so it is not a feasible or a preferred method in hospitals.

The second method is use of chlorine in the feed water for the hot water heater or hot water system. A minimum of 0.5 to 1 ppm must remain in the cold municipal water at all times for this method to work (22). The hot water is drained, and cold, chlorinated water is used to clean and rinse the system. Then, the water is heated back to the required temperature.

The third method is hyperchlorination, or shock chlorination (23). Shock chlorination involves raising chlorine levels to >2 ppm for at least 24 hours. Hot water heaters in school gyms, for example, can be drained. Cold water is then added, with 10 ppm chlorine, and left for 2 hours. The hot water heater is subsequently rinsed twice with cold water that has 1 to 3 ppm chlorine. Finally, the hot water heater is filled and the heat is turned back on. Hyperchlorination involves raising the residual chlorine in cold or room temperature water to ≥50 to 100 ppm for at least 24 hours. Whenever >10 ppm chlorine is used, special precautions should be taken to protect workers.

The fourth method involves copper-silver (Cu-Ag) ionization. This method is recognized by the Environmental Protection Agency and World Health Organization. This method works well in potable water distribution systems and the water that feeds the heat exchangers for recirculating hot waters systems in hospitals.

The fifth method is the use of chlorine dioxide or monochloramine. Chlorine dioxide can be added at 2 ppm for 6 hours. Monochloramine is more stable than chlorine. In addition, it penetrates biofilms where *Legionella* resides more effectively than chlorine. Additionally, chlorine-bromine methods are used in pools and cooling towers because bromine has a longer half-life than chlorine.

*Legionella* can also be inactivated by ultraviolet (UV) light. However, this is not a very efficient method for eliminating a bacterium that grows and reproduces in amoebae or is sheltered in corrosion particles. If UV light is used, it must be used in conjunction with another method.

Dead legs of water pipes and rubber gaskets should be eliminated, and fixture components, such as, aerators, water saver shower heads, and shower wands, should be removed and cleaned frequently. Remediators, however, should keep in mind that the organic material in environmental and even domestic water often neutralizes any disinfectants and can protect the *Legionella* biofilm.

### Who Will Do My Environmental *Legionella* Cultures?

The CDC runs a program called the ELITE (**E**nvironmental ***Legionella*** **I**solation **T**echniques **E**valuation) program (24). The program was established as a way for laboratories to test themselves and their protocols against unknown samples supplied by the CDC. Any laboratory can elect to participate in the ELITE program and can display its symbol (Fig. 2) as proof of proficiency. The CDC maintains a website (25) that lists proficient laboratories. ELITE member laboratories must recertify annually to remain on the list.

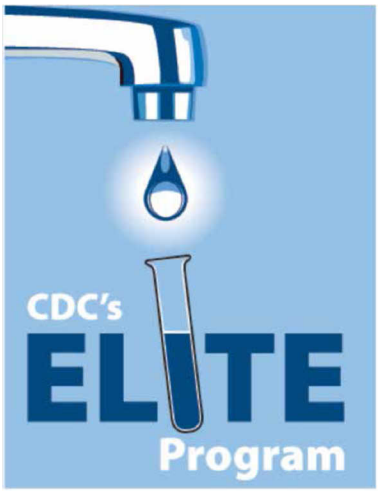


**Figure 2.** ELITE program symbol.

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