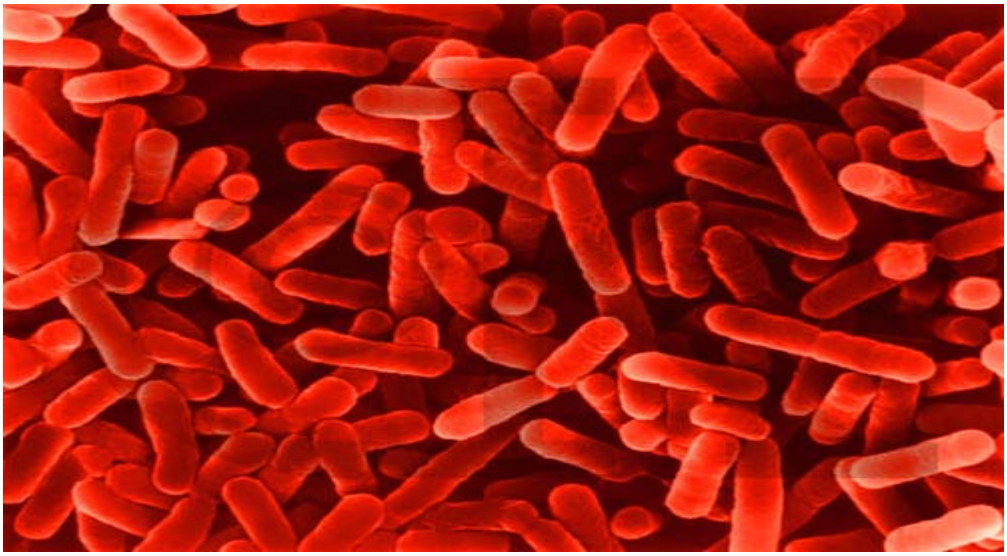
Legionella pneumophila
General Information

1. BACKGROUND

Legionella pneumophila has been identified as the leading cause of Legionnaire's Disease. This disease was first discovered in 1976 among a group of elderly men attending an American Legion Convention in Philadelphia, Pennsylvania (hence the name Legionnaire's Disease). When this outbreak first occurred it shocked the nation and the world, because no one knew why all of the men at the convention were being diagnosed with acute respiratory failure. However now, nearly thirteen years later, scientists have learned a lot about Legionnaire's Disease, as well as the *Legionella pneumophila,* the pathogen which causes it.

L. pneumophila is a common cause of nosocomial and travel-acquired pneumonia. Futhermore, *L. pneumophila* with the bacterial species *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* are the top three causes of sporadic, community-acquired pneumonia. The genus *Legionella* assigned to the family Legionellaceae. This family now includes 48 species and over 60 serogroups. Approximately 20 species are implicated in human disease. The overwhelming majority of *Legionella* infections are caused by *Legionella pneumophila*. Among all *Legionella pneumophila* serogroups, serogroup 1 is the most important causative agent.

Legionella pneumophila is a motile, rod-shaped, gram-negative, aerobic, bacterium. It is considered to be a "facultative parasite," of freshwater protozoa. This bacteria cause respiratory disease in humans when a susceptible host inhales aerosolized water containing the bacteria or aspirates water containing the bacteria.

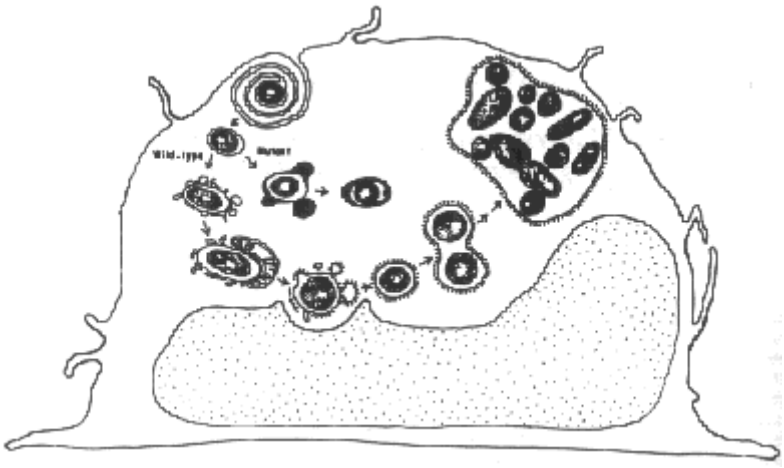


Legionella pneumophila [2]

2. CLINICAL FEATURES

A. The bacteria

Infection begins with the inhalation of the *Legionella pneumophila* bacterium. Once the Legionella reach the alveoli they come in contact with an alveolar macrophage. This only takes place, however, if the bacteria are virulent enough to overpower the host immune response. After the bacteria reach the alveolar macrophage, coiling phagocytosis begins, and the macrophage takes the bacteria into a food vacuole inside the cell. Here, *Legionella* stops the fusion of the monocyte and the lysosome. As a result, the bacteria are able to multiply inside the macrophage, where they eventually lyse the cell and infect other cells.



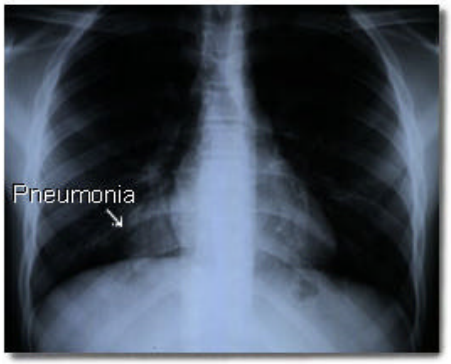
Mode of Infection [2]

B. Clinical presentation

Infection by *Legionella pneumophila* results mainly in Legionnaire's Disease. However, some strains of the bacteria have been known to manifest a disorder called Pontiac Fever. This is a very mild infection which causes influenza-like symptoms and will go away without treatment. Incidence of Legionellosis or Legionnaire's Disease have increased over the past decade or so because of the use of central air conditioning, especially in office buildings, hotels, and hospitals.

The first symptoms of Legionnaires Disease are noticed anywhere within two to ten days post-infection. On average, however, its usual incubation is about five to six days. It is primarily characterized by a bacterial pneumonia. Initially, however signs such as anorexia, malaise, myalgia, and headache should concern doctors of the possibility of infection by the *Legionella* bacterium. Other more specific symptoms of this disease are: non productive cough, high fever with chills, abdominal pain, vomiting, and diarrhea.

The majority of patients that develop Legionnaire's Disease eventually become delirious. Doctors can also diagnose this disease by looking at X-RAY films. In most cases heavy lung damage is an obvious characteristic, as well as large pockets of fluid in the alveoli. If necessary a laboratory can also analyze pieces of lung tissue. In these tissue samples scientists can look for areas of extensive lysis of phagocytotic cells where the bacteria have accumulated. The major side-effect of Legionnaire's Disease is respiratory failure which requires mechanical ventilation. Other complications are acute renal failure, hypotension, and shock.



An example of pneumonia in a young child

Anyone who has been exposed to the *Legionella pneumophila* bacterium can become ill, and develop Legionellosis or Legionnaire's Disease. However, certain people are more susceptible to the disease than others. For example, healthy young children and adults have a very small chance of becoming ill. This is because their defenses are usually in good enough condition so that the bacteria does not reach the lung, and hitherto colonize it. Here are a few of the individuals that have a likelihood of being invaded by this pathogen: elderly people (generally over the age of fifty years), males (three times more likely to infected than females), smokers, persons with immunosuppressed systems such as people who have had an organ transplant, malignancy and immunosuppression due to drugs , diabetes , chronic renal failure , people with cancer , people with the HIV virus , alcoholism

3. EPIDEMIOLOGY

A. Community Outbreaks

During the last years, the epidemiology of Legionnaires' disease has been dominated by the occurrence of several large outbreaks, two linked to cooling towers and one linked to a whirlpool spa. In April 2000, a large outbreak of Legionnaires' disease occurred among persons visiting the newly constructed aquarium in Melbourne, Australia. By June, 119 persons were confirmed to have Legionnaires' disease, and four (3.6%) persons died. This outbreak was due to a new cooling tower which had recently come on line.

In 1999, an outbreak in the Netherlands among attendees at a flower show resulted in 133 confirmed and 55 probable cases of Legionnaires' disease. Eleven percent of cases died (C. Navarro, A. Garcia-Fulgueiras, J. Kool, C. Joseph, J. Lee, C. Pelaz, and O. Tello, update on the outbreak of Legionnaires' disease in Murcia, Spain, Eurosurveillance Wkly.). This outbreak was due to a contaminated whirlpool spa on display at a consumer product fair attached to the flower show. Whirlpool spas have been implicated previously in outbreaks of Legionnaires' disease and Pontiac fever. These outbreaks involved spas that were in use. In-store whirlpool spa displays have also been implicated in the past, but never with an outbreak of this magnitude.

B. Nosocomial Outbreaks

Outbreaks of nosocomial legionellosis have been attributed to exposure of susceptible individuals to aerosols of contaminated water. Health care-associated Legionnaires disease continues to present a significant public health problem. Although the magnitude of the problem is difficult to measure, reports of outbreaks continue to abound. Hospitals represent ideal locations for Legionnaires' disease transmission: at-risk individuals are present in large numbers; plumbing systems are frequently old and complex, favoring amplification of the organism; and water temperatures are often reduced to prevent scalding of patients.

Number of cases of Legionnaires' disease reported in Europe. Since 1993, date of the cases monitoring intensification, the number of cases is in constant increasing. It has been estimated that approximately 30% of case are reported, a large number of cases are not diagnosed (mild disease, sick person rapidly be cured, Legionella not searched.....)

TABLE 1

Legionnaires' disease in Europe: total number of reported cases and incidence rate per million population, 1993 - 2006, EWGLI data

Year	Cases	No. of countries contributing data	Population (millions)	Incidence rate per million population
1993	1242	19	300	4.1
1994	1161	20	346	3.4
1995	1255	24	339	3.7
1996	1563	24	350	4.5
1997	1360	24	351	3.9
1998	1442	28	333	4.3
1999	2136	28	398	5.4
2000	2156	28	400	5.4
2001	3470	29	455	7.6
2002	4696	32	467	10.1
2003	4578	34	468	9.8
2004	4588	35	550	8.3
2005	5700	35	551	10.3
2006	6280	35	563	11.2

The CDC estimates that between 10,000 and 20,000 cases of Legionnaire's disease occur each year in the United States. Of these, 1500 to 1800 are reported to public health authorities.

4. DIAGNOSTIC

Diagnosis of legionellosis can be difficult because signs and symptoms are non-specific and not distinguish *L. pneumophila* infections from other common causes of pneumonia. *L. pneumophila* infections are considered to be fairly common but it is underdiagnosis and under-reporting. The underdiagnosis of legionellosis can in part be attributed to the need for rapid, specific and sensitive diagnostic testing methods. Laboratory diagnosis of *L. pneumophila* is typically based on either cultivation, serological, direct fluorescent antibody (DFA) staining

techniques, urinary antigen detection tests or molecular based method such as real time PCR assays.

A. Culture

Legionellae are fastidious organisms requiring cysteine and other essential growth promoting factors for their successful isolation from clinical material.

Suitable specimens for culture include bronchial washings and bronchoalvealor lavage which are the specimens of choice. When is possible, specimens should be collected before antibiotic therapy is commenced. Pleural aspirates, lung or other tissue may also be used if available. Expectorated sputum and racheal aspirates are less satisfactory as they contain relatively few legionellae and are likely to be heavely contamined with oral flora.

Legionellae usually require 48 hours incubation before growth is visible for up to five days or more. The isolation of any legionella species from clinical specimen is considered to be significant. A negative culture however does not exclude the diagnosis of legionellosis.

B. Serological test

Ideally, paired sera collected as soon as possible after the onset of illness and 3 to 6 weeks later should be tested. There may be up to nine weeks delay before seroconversion can be detected. Seroconversion is defined as a four-fold increase in the titre of antibody: against heat killed *L. pneumophila* serogroup 1 titre more or equal to 1:128.
Rem: A single high titre more or equal to 1:512 is a sensitive indicator of infection with *legionella* but may represent past infection. High titres may be present in up to 2% of the healthy population.

C. Direct Fluorescent antibody (DFA)

Microscopic examination of specimens using direct fluorescent antibody (DFA) staining was the first method used to detect legionellae in lung tissue (from autopsy or biopsy specimens) and respiratory secretions. Legionellae can be detected in respiratory secretions by DFA for several days after the start of antimicrobial therapy. DFA staining has also been used for serologic identification of *Legionella* isolates. While DFA provides a rapid method of identifying *Legionella* species, immunofluorescent microscopy is technically

demanding and should be performed only by laboratory personnel experienced in the procedure.

D. Urine Antigen Detection

Urinary antigen testing has led to the recognition of outbreaks of Legionnaires' disease and allowed a rapid public health response. In addition, urine antigen testing permits early diagnosis and initiation of appropriate antibiotic therapy. The capture antibody used in the majority of these assays is considered to be specific for *L. pneumophila* serogroup 1. Antigen is excreted as soon as 3 days after the onset of symptoms and can persist for >300 days .

Early studies on antigen detection in legionellosis patients led to the development of a commercial radioimmunoassay (RIA) for urine specimens. In studies comparing urinary antigen testing, DFA, and culture, the authors concluded that urinary antigen detection was the most useful test. A major drawback of urinary antigen testing with the RIA is the difficulty involved in the handling and disposal of radioisotopes required to perform RIA. For this reason, the RIA test was replaced by an ELISA in the mid-1980s.

But the most recent method for detecting antigenuria is the **immunochromatographic (ICT)** membrane assay. The test is simple to perform and does not require special laboratory equipment, and results can be obtained within 15 min. There are only 2 competitors in the market for the detection of antigens in urine: Binax with Now *Legionella* urinary antigen test and Coris BioConcept with the *Legionella* Respi-Strip. Both detect antigens of *L. pneumophila* serogroup 1.

The advantages of *Legionella* Respi-Strip of Coris BioConcept are described hereafter:

- ACCURATE
- SIMPLE
- RAPID
- NO INSTRUMENTATION
- SINGLE SAMPLE ANALYSIS
- MINIMAL SAMPLE MANIPULATION
- ECONOMICAL
- CE MARKED

Detection limit of the kit has been evaluated at 10 X 10^3 CFU/ml by serial dilution of *Legionella pneumophila* lysate in urine.

E. Nucleic acid based tests

PCR represents one of the few diagnostic tests with the potential to detect infections caused by all of the known species of *Legionella*. Various PCR tests that have been developed for legionellae target either random DNA sequences for *L. pneumophila*, the 5S rRNA gene, the 16S rRNA gene, or the *mip* gene. The most widely used test was a commercially produced kit designed to detect legionellae in the environment. Recently, several researchers have reported on the use of real-time PCR combined with the use of a hybridization probe to confirm the product identity for rapid detection of legionellae in clinical specimens. But a very limited number of laboratories test for legionellae by PCR at this time.

5. TREATMENT

Erythromycin is the antibiotic currently recommended for treating persons with Legionnaires' disease. In severe cases, a second drug, rifampin, may be used in addition. Other drugs are available for patients unable to tolerate erythromycin. Delay in starting appropriate therapy has been associated with increased mortality.

Pontiac fever requires no specific treatment.

6. TRANSMISSION

Outbreaks of legionellosis have occurred after persons have breathed mist that come from a water source (e.g., air conditioning cooling towers, whirlpool spas, showers) contamined with *Legionella* bacteria. Persons may be exposed to these mists in homes, workplaces, hospitals, or public places. Legionellosis is not passed from person to person, and there is no evidence of persons becoming infected from auto air conditioners or household window air-conditioning units.

7. Prevention

Most outbreacks reported to date have been associated with cooling towers, evaporative condensers of air-conditioning devices, potable water at elevated temperatures (especially in hospitals and hotels), hot whirpool and spa baths, nebulizers, and certain potting composts. The greatest risk seems to be associated with water subjected to prolonged periods of stagnation and in systems that frequently maintained at temperatures of 25-50 °C; this range of temperatures should therefore be avoided as far as possible.

Water systems-particularly cooling towers and evaporative condensers should be designed, constructed, and operated in such a way that microbial growth is minimized. High water temperature is the most efficient approach to both intermittent disinfection and continuous control. In hot-water distribution systems, water temperatures should exceed 60°C in boilers, reservoirs, and circulating pipes, and reach 50°C at outlets. Continuous surveillance and disinfection have been proposed for water systems in hospitals and in public swimming pools, hot whirlpool and spa baths and the like, and for medical and dental equipment that uses water. However, opinion continues to be divided on this issue (Centers for Disease Control and Prevention, 1997), and there is no generally accepted threshold limit for the concentration of *legionella* in water. Surveillance of the drinking-water and hot-water supply systems in hospitals is recommended by some (Allegheny County health Department, 1997), and considered prudent in institutions for the elderly and, possibly, in large hotels.

Total prevention of sporadic infections is impossible, because of the widespread occurrence of *Legionella* in all environments. In hospitals, however, all clinical specimens from patients with symptoms of pneumonia should be examined for *Legionella, Legionella* antibodies and *Legionella* antigen and every effort must be made to identify the source of infection and implement measures to interrupt transmission. Transplatant patients should be scrupulously protected from exposure to *Legionella* antibodies during immonosuppression; their drinking water should be sterilized, and sterilized water should be used for washing these patients.

8. BIBLIOGRAPHIC REFERENCES

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