Legionella

Legionella is a pathogenic Gram negative bacterium, including species that cause legionellosis (Legionnaires' disease), most notably *L. pneumophila* [1]. It may be readily visualized with a silver stain.

It is common in many environments, with at least 50 species and 70 serogroups identified. The side-chains of the cell wall carry the bases responsible for the somatic antigen specificity of these organisms. The chemical composition of these side chains both with respect to components as well as arrangement of the different sugars determines the nature of the somatic or O antigen determinants, which are essential means of serologically classifying many Gram-negative bacteria.

Legionella colonies growing on BCYE agar with L-cysteine

Scientific classification

Domain: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Legionellales

Family: Legionellaceae

Genus: *Legionella*

Legionella sp. under UV illumination

Virulence factors

Legionella live within amoebae in the natural environment [2]. *Legionella* species are the causative agent of the human Legionnaires' disease and the lesser form, Pontiac fever. Individual biological and immunological factors mediating virulence have not been explicitly defined [3].However, analysis of the infection process in protozoa and

human host cells has identified certain general factors that may affect virulence, such as:

- expression of multiple proteins during infection of macrophages [4].
- expression of certain proteases [5] the proteases are thought to be important in the pathogenicity of *L. pneumophila*, but it is not clear whether they contribute to virulence.
- plasmids contained in *L. pneumophila*, which may affect intracellular survival [6].

One product of Legionella clearly associated with virulence is the 24-kDa macrophage infectivity potentiator (Mip) protein, coded for by the mip gene [7].The Mip protein is thought to be conserved throughout the genus [8]; it is required for efficient infection of both mammalian phagocytic cells and protozoa [9], but its mechanism of action is unknown. The type IV secretion system, a bacterial conjugation system used for transporting and injecting DNA or toxins into target cells, has a crucial role in the spread of pathogenicity. Within the loci encoding the type IV secretion systems (dot/icm) are 24 genes essential for infection of the host cell, and involved in assembling and activating conjugal transfer of plasmid DNA. *L. pneumophila* uses these operons to deliver virulence factors and a protein that diverts the phagosome from its endocytic pathway [10]. Genes such as pilE (coding for the pilin protein) and pilD (coding for prepilin peptidase) are important for unrestricted

intracellular growth. Other loci involved in intracellular multiplication are mak (macrophage killing), mil (macrophage-specific infectivity loci), and pmi (protozoan and macrophage infectivity). Defects in any of these loci obstruct or interrupt intracellular growth [10].

Tissue-destructive protease is another important factor in the ability of Legionella to cause infection. Other factors that may increase virulence include several cytotoxins, heat shock proteins and compounds associated with iron uptake. The stationary phase response and the iron acquisition functions of *L. pneumophila* also play key roles in pathogenesis, as do a number of other loci, including the pts and enh genes [11].

Virulence factors affect the ability of legionellae to grow within protozoa, as seen from studies showing the effect of incubating a virulent *L. pneumophila* strain and the corresponding avirulent strain with an *Acanthamoebae polyphaga* from a source implicated in an outbreak of Legionnaires' disease [12].

Transmission

An infected source (e.g. a fountain) can disseminate sprays or droplets of water containing legionellae, commonly referred to as aerosols. When this occurs, most or all of the water in the droplet evaporates quickly, leaving airborne particulate matter that is small enough to be inhaled. Particles of less than 5 μm in diameter can be

deeply inhaled, and enter the respiratory airways to cause legionellosis [13].

Colorized SEM of *Legionella pneumophila* bacteria

Legionella infections have frequently been associated with sources at distances of up to 3.2 kilometres [14]; recent evidence suggests that infection may be possible at even longer distances [15]. There is evidence that virulence is an important factor in the survival of Legionella in aerosols, with the most virulent strains surviving longer than their less virulent counterparts [16].

There is no evidence of person-to-person transmission of either Legionnaires' disease or Pontiac fever [17].

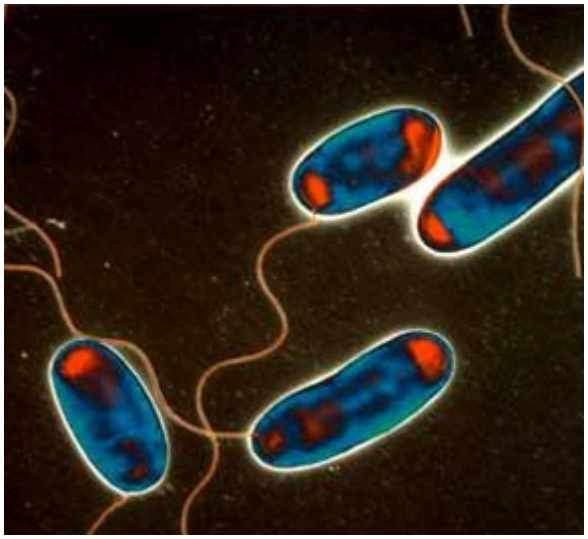
Once inside a host, incubation may take up to two weeks. Initial symptoms are flu-like, including fever, chills, and dry cough. Advanced stages of the disease cause problems with the gastrointestinal tract and the nervous system and lead to diarrhea and nausea. Other advanced symptoms of pneumonia may also present.

However, the disease is generally not a threat to most healthy individuals, and tends to lead to harmful symptoms only in those with a compromised immune system and the elderly. Consequently, it should be actively checked for in the water systems of hospitals and nursing homes. The Texas Department of State Health services provide recommendations for hospitals to detect and prevent the spread of nosocomial infection due to *Legionella* [18]. According to the journal "Infection Control and Hospital Epidemiology," Hospital-acquired *Legionella pneumonia* has a fatality rate of 28%, and the source is the water distribution system [19].

Table 1. Main characteristics of Legionnaires' disease and Pontiac fever*

Characteristic	Legionnaires' disease	Pontiac fever
Incubation period	2–10 days, rarely up to 20 days	5 hrs–3 days (most commonly 24–48 hrs)
Duration	Weeks	2–5 days
Case–fatality rate	Variable depending on susceptibility; in hospital patients, can reach 40–80%	No deaths
Attack rate	0.1–5% of the general population 0.4–14% in hospitals	Up to 95%
Symptoms	• Often non-specific • Loss of strength (asthenia) • High fever • Headache • Nonproductive, dry cough • Sometimes expectoration blood-streaked • Chills • Muscle pain • Difficulty in breathing, chest pain • Diarrhoea (25–50% of cases) • Vomiting, nausea (10–30% of cases) • Central nervous system manifestations, such as confusion and delirium (50% of cases)	• Influenza-like illness (moderate to severe influenza) • Loss of strength (asthenia), tiredness • High fever and chills • Muscle pain (myalgia) • Headache • Joint pain (arthralgia) • Diarrhoea • Nausea, vomiting (in a small proportion of people) • Difficult breathing

	• Renal failure • Hyponatraemia (serum sodium <131 mmol/litre) • Lactate dehydrogenase levels >700 units/ml • Failure to respond to beta-lactam antibiotics or aminoglycosides • Gram stain of respiratory specimens with numerous neutrophils and no visible organisms	(dyspnoea) and dry cough



Legionella bacteria

Species

L. adelaidensis
L. anisa
L. beliardensis
L. birminghamensis
L. bozemanii
L. brunensis
L. busanensis
L. cherrii
L. cincinnatiensis
L. donaldsonii
L. drancourtii
L. drozanskii
L. erythra
L. fairfieldensis
L. fallonii
L. feeleii
L. geestiana
L. genomospecies
L. gratiana
L. gresilensis
L. hackeliae
L. impletisoli
L. israelensis
L. jamestowniensis
‘Candidatus *Legionella jeonii’*
L. jordanis
L. lansingensis
L. londiniensis
L. longbeachae
L. lytica
L. maceachernii
L. micdadei
L. moravica

L. nautarum
L. oakridgensis
L. parisiensis
L. pneumophila
L. quateirensis
L. quinlivanii
L. rowbothamii
L. rubrilucens
L. sainthelensi
L. santicrucis
L. shakespearei
L. spiritensis
L. steigerwaltii
L. taurinensis
L. tucsonensis
L. wadsworthii
L. waltersii
L. worsleiensis
L. yabuuchiae

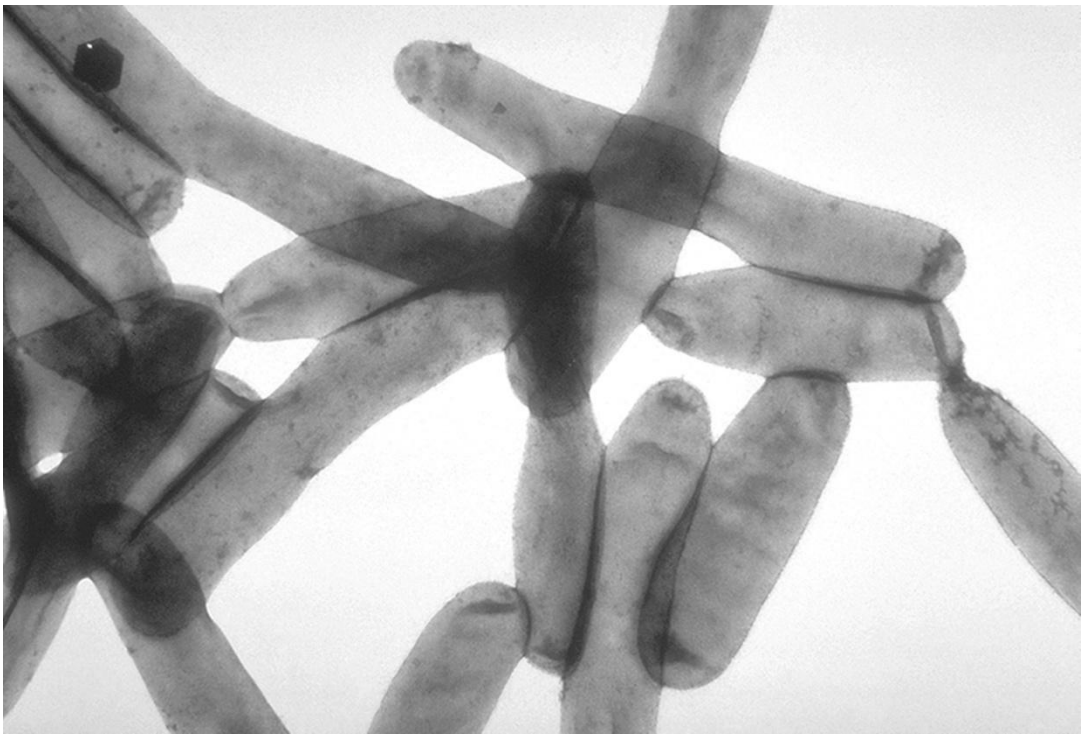
Selected species: *L. pneumophila*; *L. longbeachae*

1. *Legionella pneumophila*

Legionella pneumophila is a thin, aerobic, pleomorphic, flagellated, non-spore forming and Gram-negative bacterium of the genus Legionella [20]. *L. pneumophila* is the primary human pathogenic bacterium in this group and is the causative agent of legionellosis or Legionnaires' disease.

L. pneumophila is an acid-fast, non-sporulating, and morphologically a non-capsulated, rod-like bacterium, often

characterized as being a coccobacillus. Aerobic and unable to hydrolyse gelatin or produce urease, it is also non-fermentative. *L. pneumophila* is neither pigmented nor does it autofluoresce. It is oxidase- and catalase-positive, and produces beta-lactamase. *L. pneumophila* has a colony morphology that is gray-white with a textured, cut-glass appearance; it also requires cysteine and iron to thrive.



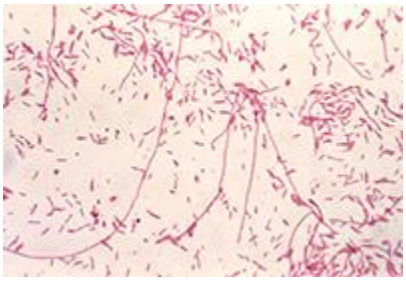
TEM image of *L. pneumophila*

Detection

Sera have been used both for slide agglutination studies as well as for direct detection of bacteria in tissues using fluorescent-labelled antibody. Specific antibody in patients can be determined by the indirect

fluorescent antibody test. ELISA and microagglutination tests have also been successfully applied.

Legionella stains poorly with gram stain, stains positive with silver, and is cultured on charcoal yeast extract with iron and cysteine.



Gram-stained micrograph of *Legionella pneumophila* bacteria

Virulence factors

In humans, *L. pneumophila* invades and replicates in macrophages. The internalization of the bacteria can be enhanced by the presence of antibody and complement, but is not absolutely required. A pseudopod coils around the bacterium in this unique form of phagocytosis. Once internalized, the bacteria surround themselves in a membrane-bound vacuole that does not fuse with lysosomes that would otherwise degrade the bacteria. In this protected compartment, the bacteria multiply. The bacteria use a Type IVB secretion system known as Icm/Dot to inject effector proteins into the host. These effectors are involved in increasing the bacteria's ability to survive inside the host cell. Survival is enhanced by these effector proteins (Ank proteins) because they interfere with

fusion of the Legionella-containing vacuole with the host's degradation endosomes [21].

Symptoms

Symptoms of Legionnaires' disease tend to get worse during the first 4 - 6 days. They typically improve in another 4 - 5 days.

Symptoms may include: Chest pain, coughing up blood, fever, gastrointestinal symptoms, such as diarrhea, nausea, vomiting, and abdominal pain, general discomfort, uneasiness, or ill feeling (malaise), headache, joint pain, lack of coordination (ataxia), loss of energy, muscle aches and stiffness, nonproductive cough, shaking chills and shortness of breath [22].

Treatment

Antibiotics are used to fight the infection. Treatment is started as soon as Legionnaires' disease is suspected, without waiting for confirmation by lab test.

- Antibiotics commonly used to treat this condition include:
  1. Quinolones (ciprofloxacin, levofloxacin, moxifloxacin, or gatifloxacin).
  2. Macrolides (azithromycin, clarithromycin, or erythromycin).
- Other treatments may include:
  1. Fluid and electrolyte replacement.

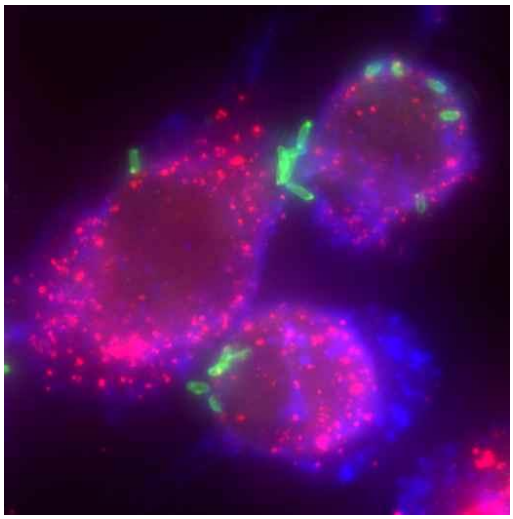
2. Oxygen (given through a mask or breathing machine).

Prevention

Treating water delivery systems can prevent the spread of disease [23].

2. *Legionella longbeachae*

Legionella longbeachae is one species of the family Legionellaceae. It was first isolated from a patient in Long Beach, California. It is found predominantly in soil and potting compost. In humans, the infection is sometimes called **Pontiac Fever**. The infection can be very serious, often leading to hospitalization [24] and sometimes death [25].



Legionella longbeachae

Virulence factors

As described before *Legionella sp.* invades and replicates in macrophages. The internalization of the bacteria can be enhanced by the presence of antibody and complement, but is not absolutely required. A pseudopod coils around the bacterium in this unique form of phagocytosis.

Transmission

Like other *Legionella* species, person-to-person transmission has not been documented. However, unlike other species the primary transmission mode is inhalation of dust from contaminated compost or soil that contains the organism causing legionellosis [26].

Modes of transmission include poor hand-washing practices after gardening, long-term smoking and being near dripping hanging flower pots. Awareness of a possible health risk with potting mix protected against illness. Inhalation and ingestion are possible modes of transmission. Exposure to aerosolized organisms and poor gardening hygiene may be important predisposing factors to *L. longbeachae* infection [27].

Symptoms

Early symptoms include fever, chills, headache, shortness of breath, sometimes dry cough, muscle aches and pain [28].

Most people who breathe in the bacteria do not become ill. The risk of disease is increased with age, smoking, and in people with weakened immune systems [28].

Treatment

The list of treatments mentioned in various sources for *Legionella longbeachae* infection includes the following;

- Antibiotics (Intravenous antibiotics, Erythromycin, Azithromycin, Ciprofloxacin, Ofloxacin, Levofloxacin, Doxycyclin and Trimethoprim-sulfamethoxazole),
- Oxygen therapy,
- Intravenous fluid therapy,
- Hospitalization,
- Intensive care admission - dependant upon severity of illness, and
- Ventilatory support - dependant upon severity of illness.

Prevention

Compost must be handled with care, damp down with water to reduce dust before handling, use a face mask covering nose and mouth

to reduce the risk of inhaling the dust, especially for those at high risk from infection.

Studies advocate the introduction of an industry standard that ensures the use of face masks when handling potting mix and attaching masks and warning labels to potting mix bags sold to the public [29].

It is noteworthy that compost packaging in Australia has a *L. longbeachae* warning label. The New South Wales state government recommends that people reduce exposure to potting mix dust by following the manufacturers' warning present on potting mix labels, including:

1. Wetting down the potting mix to reduce the dust.
2. Wearing gloves and a P2 mask when using potting mix.
3. Washing hands after handling potting mix or soil, and before eating, drinking or smoking [30].

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