Bacterial Pathogenesis of *Legionella*

Carissa Cabrera

Case 3

To celebrate Tom's retirement his wife and two adult children accompany him on a long anticipated cruise. Tom's asthma flares up a few days before the cruise but with a corticosteroid nebulizer he feels well enough to join the cruise. Even more than the rest of his family, Tom enjoys the various hot tubs aboard the massive ship those first few days, relishing the relaxation after a busy final year at work. On the fifth day of the cruise, Tom wakes up in a sweat with a cough that continues throughout the day. As the day wears on he feels worse with a headache, muscle aches and nausea accompanying the cough. His wife arranges for the cruise doctor to visit him in his cabin. The doctor examines Tom, notes his high temperature, nonproductive cough and recent history of asthma and corticosteroid therapy. She takes a full history including taking note of his activities during the first days of the cruise and diagnoses Tom with pneumonia. She starts Tom on azithromycin. By the time the ship returns to port two days later, 5 more people have been diagnosed with a similar pneumonia, several of whom have a slightly compromised immune system, as Tom does. One of the others is admitted to hospital, where sputum and urine samples are tested and reveal a diagnosis of Legionellosis. Public health authorities are notified and the ship takes extra time in port to allow for an enhanced cleaning to be performed on all of the hot tubs.

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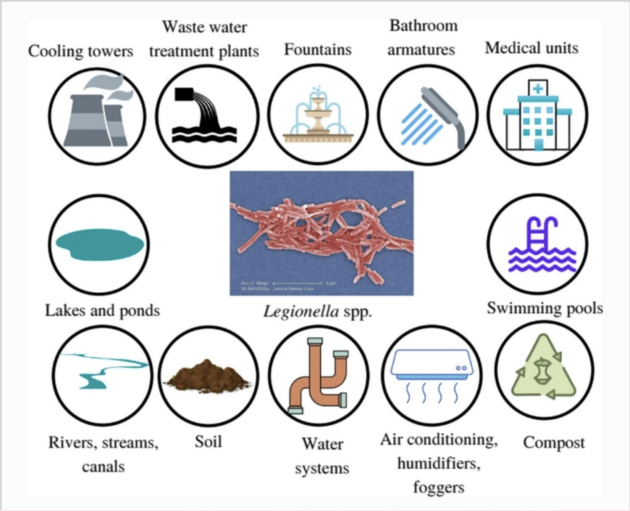
INTRODUCTION

Legionella is a Gram-negative bacilli. It is aerobic, waterborne, and nonmotile. It also tests positive for oxidase and catalase (1)

https://clubsolutionsmagazine.com/2020/08/reduce-legionella-bacteria-after-covid-19-shutdown/

Encounter

- *Legionella* is found in aquatic environments (2)
  - Natural habitats are fresh reservoirs, watercourses, moist soil, and compost material (2)
- It is also seen in water systems (2)
  - Plumbing systems, air-conditioning units, bathtubs, and showers (2)



https://link.springer.com/article/10.1007/s11356-022-22950-9

Geographical Distribution around the World

- 864 reported cases made 1,279 visits to accommodation sites around the world (3)
- A total of 66 countries were visited in 2-10 days before the onset of disease (3)
- 654 cases travelled in the EU: 621 cases visited only one Member State and 33 more than one (3)

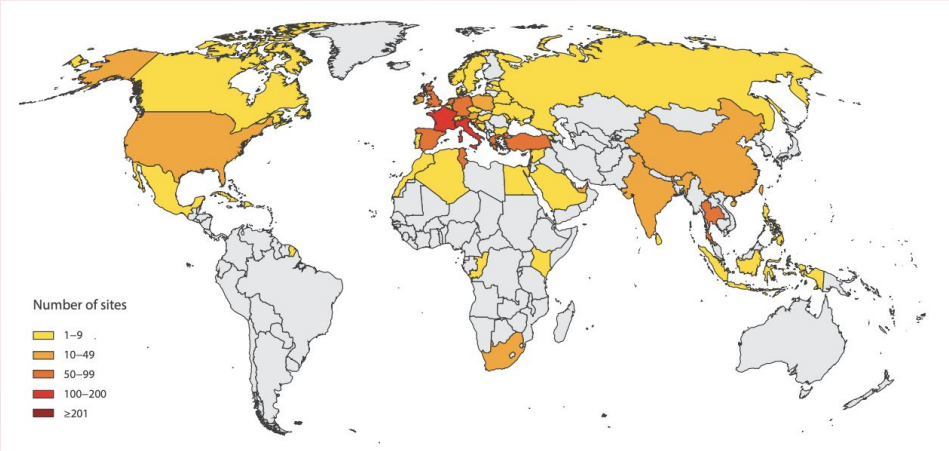


Figure 1: Accommodation sites per destination country associated with cases of travel-associated Legionnaires; disease world wide, 2010 (3)

Geographical Distribution in Europe

- Italy had the most infected cases, n=209 (3)
- Then Spain had 177 cases, France had 172 cases, and Turkey had 48 cases (3)
- 169 cases were French residents (3)

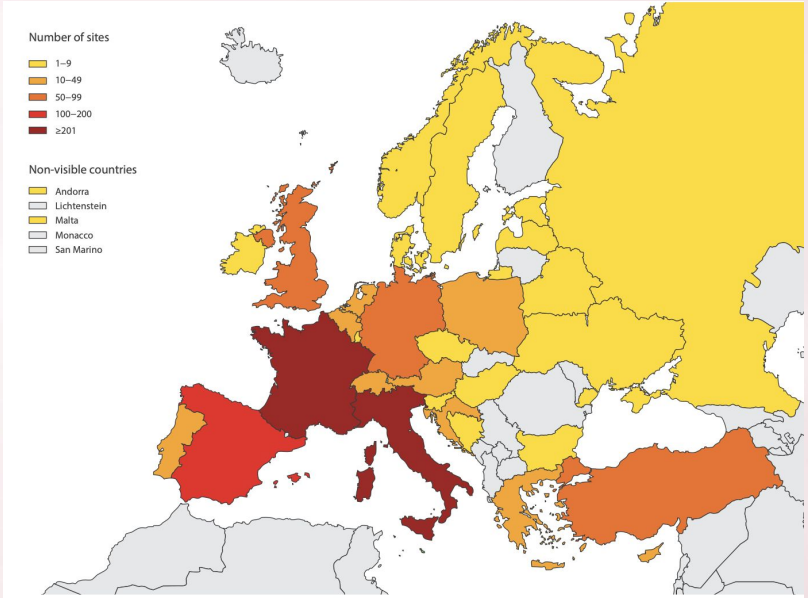
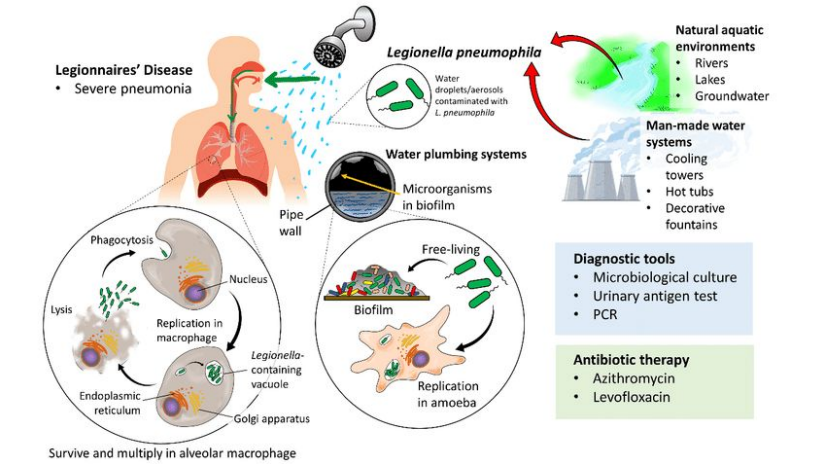


Figure 2: Accommodation sites per destination country associated with cases of travel-associated Legionnaires' disease, EU Member States and neighbouring countries, 2010 (3)

Entry

- *Legionella* is transmitted through human aerosols (4)
  - It can also infect someone via consumption or direct exposure to contaminated water (4)
  - In Tom's case, he may have been more susceptible to the disease as his immune system was compromised due to his asthma acting-up

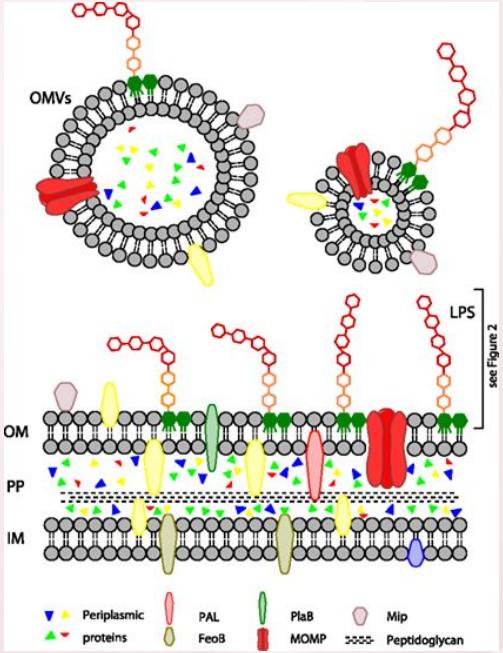


https://www.researchgate.net/figure/The-transmission-sources-life-cycle-within-water-systems-and-human-macrophage-the_fig1_350761825

Virulence Factors

- *Legionella* has surface virulence factors that increase its infectivity (5)
  - Lipopolysaccharide, flagella, pili, and outer membrane proteins (5)

- Flagella is used to improve the bacterias chances of being taken up into the cell (6)
- LPS plays a vital role in interacting with host cells and manipulating intracellular trafficking (7)
  - Unusual structure of lipid A helps the bacteria from being recognized by the hosts innate immune system (7)
- It has a type IV pili (5)
  - It enhances the bacteria's ability to colonize in the lung tissue (5)



https://www.frontiersin.org/articles/10.3389/fmicb.2011.00074/full

Outer Membrane Proteins

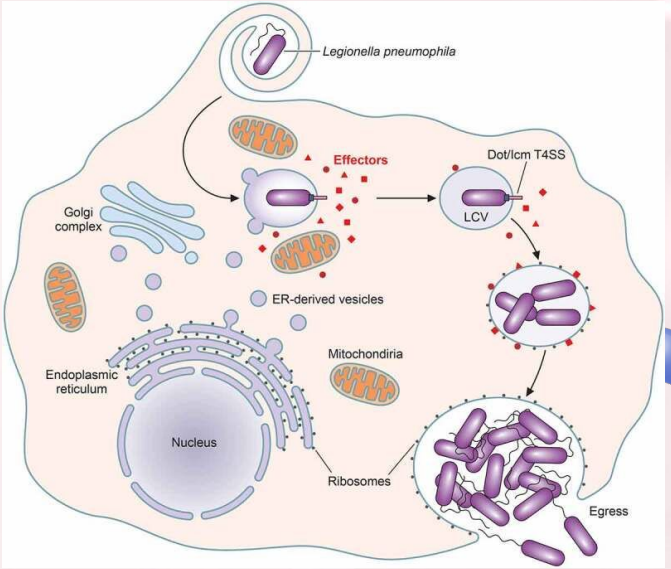
- *Legionella* has a variety of membrane proteins that contribute to adherence and entry

- *rtxA*
  - Involved in adherence, cytotoxicity, and pore formation (8)
- LcI
  - Adhesin that enhances interactions with lung epithelia (9)
  - It also plays a vital role in biofilm formation (9)
- LadC
  - *Legionella pneumophila* exclusively uses this protein (10)
  - It produces cAMP, this will manipulate protein-protein interactions and control protein activities (10)

Multiplication

- *Legionella* is phagocytosed by human alveolar macrophages once it is inhaled by the host via aerosol droplets (11)
  - Once taken up by macrophages it will lose its flagella and form a membrane-bound compartment called a *Legionella*-containing vacuole (LCV) (11)
  - The LCV will escape the endocytic pathway (11)
    - This is a result of its Dot/icm type IV secretion system (11, 12)
    - It will translocate ~300 effector proteins into the host cell (11)
  - LCV will be covered with ribosomes and intracellular replication will occur (11)
  - Once host cells nutrients are scarce, *Legionella* will be flagellated and egress the cell (11)

Figure 3: Life cycle of *Legionella* in the Host Cell



https://www.tandfonline.com/doi/full/10.1080/21505594.2021.1903199

Dot/icm Type IV Secretion System

- Contains ~27 different components (12)
- It has a core complex made up of 5 proteins: DotC, DotD, DotF, DotG, DotH (12)
- Proteins injected, such as EnhC, LpnE, LvhB2, and HtpB play an important role in bacterial entry (13)
- It will modify host cell processes (14)
  - Membrane transport systems (14)
  - Inhibit host cell apoptosis (14)
  - And change host cell signalling pathways (14)

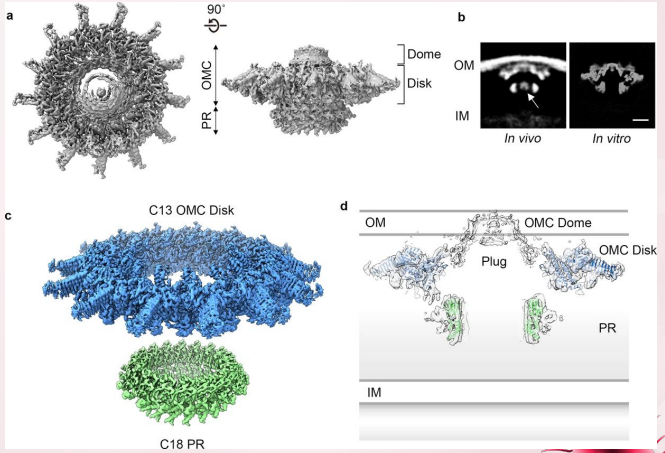
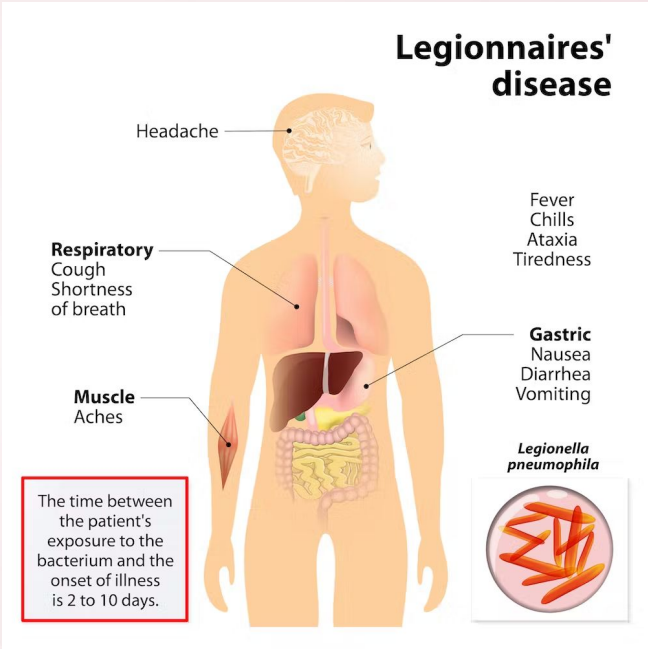


Figure 4: Cryo-EM structure of the *L. pneumophila* Dot/Icm T4SS. https://elifesciences.org/articles/59530

Spread

- When the bacteria is released after replication, *Legionella* can migrate across the epithelium of the lungs, this can lead to complications (15):
    - Bacteremia spread, invasion of different types of organs, and septic shock (15)



https://theconversation.com/why-are-people-still-dying-from-legionnaires-disease-121862

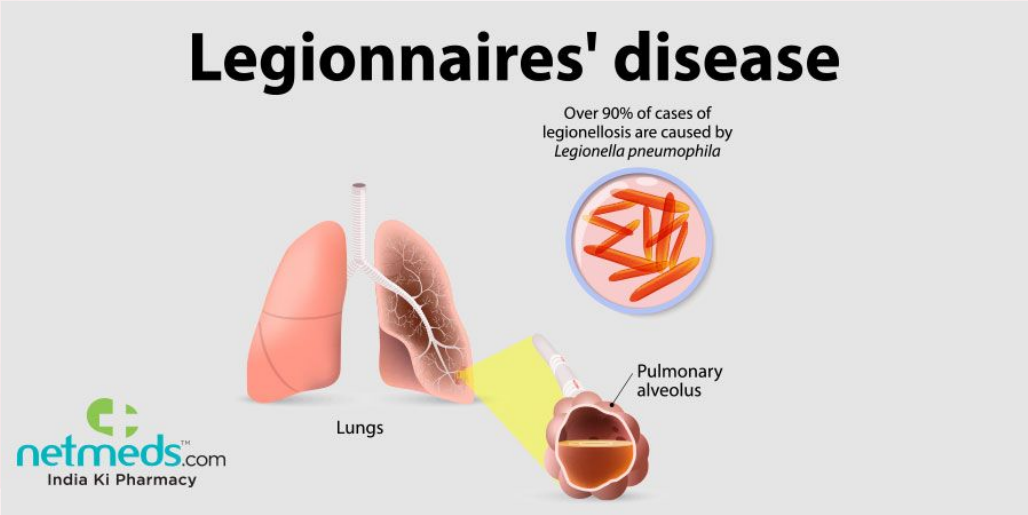
Bacterial Damage

- Neutrophils and monocytes mediate alveolar tissue
  - Infection can produce an inflammatory infiltrate of neutrophils and macrophages (16)
    - This can result in cell necrosis, abscess formation, and small blood vessel inflammation (17)
    - Inflammation can lead to symptoms such as cough, fever, and chest pain (17)

https://my.clevelandclinic.org/health/diseases/17750-legionnaires-disease

Bacterial Damage

- Toll-like receptors can also activate reactive oxygen species, NFκB activation of pro-inflammatory cytokine, type I interferon (IFN) (18)
    - These different factors produce tissue damage in lungs which destroys airspaces and disrupts gas exchange in the lungs (16, 18)



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