An Insight into the Microbiology, Epidemiology, and Host Cell Biology of Legionella Pneumophila: A Review of Literature

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Legionnaires’ disease (LD) is a type of severe pneumonia that mainly caused by bacteria of the genus Legionella. LD bacteria reside in the water systems of facilities where lack of water exchange or flow plays a crucial role in enhancing bacterial growth. The under-recognition of the dangers of Legionella along with easing of Coronavirus disease 2019 (COVID-19) lockdown restrictions and global reopening, pose a potential increased risk of developing LD. Various Legionella species can lead to legionellosis infections, including LD and Pontiac fever. Legionellosis cases is generally found in natural or artificial aquatic environments such as cooling towers, hot water tanks, or air conditioning. The bacteria elude the host’s immune responses by various strategies, including releasing effector proteins. Thus, this review provides insight into the microbiology, epidemiology, and host cell biology of L. pneumophila, as well as an emphasis on the bacterial novel survival strategies of L. pneumophila. Also, suggests taking intensive actions towards closed buildings as a potential source of bacterial infection.

Keywords: COVID-19 restrictions; Epidemiology; Legionnaires' disease; Legionella pneumophila; T4SS, LCV

L. pneumophila is an aerobic gram-negative, flagellated, rod-shaped, intracellular waterborne bacterium of the Legionella genus and the causative agent of most LD cases (see Figure 1). Legionella was the first defined bacterium that intracellularly multiplied within protozoan (initially aquatic amoebae), that help in understanding the bacteria’s capacity to infect protozoa. This bacterium is ubiquitous, usually found in moist soil and water, freshwater systems are the main reservoir of L. pneumophila1 Although freshwater systems colonised by Legionella can disperse aerosols through showers, whirlpools, fountains, and cooling towers, L. pneumophila prefers to grow in hot water systems, including hot water tanks and hot tubes. Legionella can proliferate in many types of niches. It can live in planktonic form, co-existing mainly within multi-organismal biofilms or...
replicating inside an amoebae within a freshwater system. This commonly leads to influenza-like outbreaks caused by different Legionella species\(^2\). The inhalation of Legionella micro-aspiration results in both LD and a mild respiratory illness called Pontiac fever\(^3\). LD manifests as a pneumonia illness with a case fatality of almost 10%. Non-fatal Pontiac fever is a less severe flu-like illness caused by L. pneumophila, with symptoms of fever, chills, and headache\(^4\). Those most at risk of LD include the elderly, smokers, patients with chronic lung diseases (such as emphysema), and immunocompromised individuals (e.g. people with cancer or kidney failure\(^4\)).

Since the disease was first identified in 1976, the highest number of LD outbreaks has been recorded in 2018, with approximately 10,000 cases in the US according to the CDC, different outbreaks have also been reported in such global locations as Canada, the United Kingdom, Italy, Sweden, Portugal, and Japan\(^6-9\). In general, the bacteria can cause both population and nosocomial pneumonia, with sporadic cases accounting for 85%. Legionella species are responsible for up to 50% of cases of hospital-acquired pneumonia\(^10\). Recently, COVID-19 lockdown restrictions have raised concerns about water stagnation and the consequent facilitation of bacterial growth\(^11, 12\). Thus, controlling microbial infections through water quality monitoring in closed buildings is recommended. This article will review the literature associated with the study of L. pneumophila, including its microbiological and epidemiological features. The article will also briefly describe the host biology and pathogenicity mechanisms of the bacteria.

L. pneumophila

In 1976, more than 200 people developed mysterious severe pneumonia illnesses resulting in 34 deaths\(^1-13\). Since this outbreak’s causative agent was unknown, the US Centers for Disease Control and Prevention (CDC) investigated the source of the infection. Although the source of Legionella was then unknown, investigators hypothesised that the air conditioning of the hotel in which the patients had stayed was the source of the infection. The CDC and the US National Institutes of Health (NIH) were the first medical organisations to start researching LD, with CDC beginning one of their largest investigations to follow the source of the outbreak. This epidemiological investigation aimed to track the etiological agent using various laboratory techniques. In 1977, the microbiologist Joseph McDade discovered L. pneumophila as the etiological agent of the LD outbreak\(^14\). He described it as a rod-shaped, Gram-negative bacterium and named it after the members of the American Legion first affected with the illness\(^15, 16\). Two years later, another outbreak occurred in which investigators found that a hospital’s air conditioning cooling tower was the source of L. pneumophila\(^17, 18\).

L. pneumophila is a facultative intracellular pathogen. It replicates within human alveolar macrophages to avoid phagolysosome fusion and maintain replication within the host cell\(^19\). The intracellular vesicle of the bacteria thus becomes vigorously motile and overwhelmed by the infection. Consequently, lysis of the host cell releases the bacterial progeny from the macrophage to the surrounding environment\(^19\). This increases the patient’s susceptibility to acute lung inflammation, particularly in immunocompromised people and the elderly. Following inhalation of the aerosols, L. pneumophila avoids degradation and controls the immune system to form Legionella Containing Vacuoles (LCV). The LCV then employ rough endoplasmic reticulum and mitochondria to support L. pneumophila intracellular replication. Additionally, L. pneumophila has developed mechanisms such as hijacking host cell functions by secreting effector proteins using unique secretion systems\(^20\). Effector proteins of L. pneumophila have exceeded 300 effectors. These proteins facilitate bacterial survival primarily through the acquisition of the host’s nutrients. The excessive activity of effector proteins, paradoxically, increases pro-inflammatory cytokines\(^21, 22\). In addition to effectors, virulence factors including flagella, type IV, and LPS play a role in enhancing L. pneumophila pathogenesis\(^23, 24\).

Different species of Legionella have been associated with both community-acquired pneumonia and nosocomial pneumonia\(^25\). Furthermore, Legionella is generally motile and requires specific environmental conditions to grow, including the presence of cystine and iron. L. pneumophila replicates intracellularly within eukaryotic host cells such as protozoa and macrophages\(^24, 26\). The bacteria replicate in the human lung, where alveolar macrophages lead to...
phagocytosis of the bacteria after the inhalation of contaminated water aerosols\textsuperscript{27}. Alveolar macrophages are thus considered the primary type of human cell associated with this infection. Moreover, the ability of \textit{L. pneumophila} to adapt to different hosts and to infect humans is due to its high-volume acquisition of effector proteins and genes\textsuperscript{30}. The genus \textit{Legionella} have surpassed 60 species and more than 70 serogroups\textsuperscript{28}, and the numbers continue to increase\textsuperscript{29}. Thirty serogroups have been successfully isolated from patients and associated with human disease\textsuperscript{30}. Whereas \textit{L. pneumophila} was found to be responsible for most LD cases (approximately 95\%) compared to other \textit{Legionella} species. The strain associated with nearly 84\% of LD cases is serogroup one, found in natural habitats, followed by \textit{L. longbeachae} (3.9\%)\textsuperscript{31}. These strains’ high virulence is due to various ecological and physiological features, such as the O-antigen proteins identified in serogroup one\textsuperscript{32}. In addition, serogroups four and six are also associated with the disease\textsuperscript{28, 33}.

**Mode of transmission**

Despite the severe outcomes of this disease, there is limited evidence of human-to-human transmission of \textit{L. pneumophila}\textsuperscript{34}. Consequently, this pathogen is known as ‘an accidental pathogen’ for which humans are the last host meaning that there is no subsequent transmission\textsuperscript{37}. One mode of transmission for \textit{L. pneumophila} to humans is through the inhalation of contaminated water droplets. The bacteria can reside and grow in artificial water systems, such as pipes, to form a biofilm\textsuperscript{35}. Thus, \textit{L. pneumophila} can cause disease only if it is inhaled or aspirated\textsuperscript{36}. Serogroup one of \textit{L. pneumophila} has been found across the United States, in 47\% of cold-water of the publicly-used taps\textsuperscript{37}, followed by multiple LD’s outbreaks that have been related to several sources including contaminated cooling towers\textsuperscript{38}, closed-water distribution systems, and public whirlpool spas\textsuperscript{39}. Other mechanisms and settings include hospital equipment, air conditioning, hotels, and cruise ships.

LD cases have also found to be associated with supermarket mist machines, fountains, and ice machines\textsuperscript{40}. As the wide range of these settings makes clear, any aerosol generation source can transmit \textit{Legionella}. Although \textit{Legionella} antibodies have been found in animal sera, zoonotic transmission has not yet been detected\textsuperscript{41}. However, co-infection may arise, particularly in immunocompromised patients\textsuperscript{52}. Additionally, micropinocytosis plays a critical role in \textit{L. pneumophila} pathogenicity; this process produces macropinosome, a vesicle generated from fusion of the membrane projections\textsuperscript{33, 44}. It has been found that phospholipids such as phosphatidylinositol-3-kinase (PI3K) are involved in macropinosome formation\textsuperscript{45}. Although the entry mechanisms of such protein’s remain unclear, it is important to mention that various structural genes, including \textit{RtxA} and \textit{enhC}, have a crucial role in facilitating pathogen transmission and attachment to host cells\textsuperscript{46}. For instance, protein-protein interaction is facilitated by Sel1-like repeat (SLR), which is encoded by \textit{enhC}\textsuperscript{47}. Furthermore, Ca\textsuperscript{2+} binding is mediated by \textit{RtxA}, which produces a total of eight motifs\textsuperscript{48, 49}.

**Metabolic pathway**

\textit{L. pneumophila} replicates in both free-living amoebae and a host’s respiratory tract macrophages within LCV\textsuperscript{50}. The formation of LCV, which are endoplasmic reticulum (ER)-associated compartments, involves a complex process\textsuperscript{51}. That requires the bacteria to employ more than 300 effector proteins, including Defective Organelle Trafficking/Intracellular Multiplication (Dot/Icm) and to be translocated into the host’s cell by T4SS\textsuperscript{52}. The wide variety of free-living protozoa explains why that \textit{L. pneumophila} the most significant number of effector proteins compared to other bacteria. During replication, the membrane-bound compartment LCV protects the bacteria by preventing lysosomal degradation\textsuperscript{53}. Also, within different ecological niches, the survival of \textit{L. pneumophila} is attributed to the ability of LCV to facilitate the uptake of nutrients in the infected host cells\textsuperscript{51}. In free-living protozoa, where the amino acids are the preferred carbon source for \textit{L. pneumophila}\textsuperscript{54}.

\textit{L. pneumophila} within the host cell can employ amino acid transporters to uptake the host amino acids as sources of carbon and energy. The effectors that \textit{L. pneumophila} utilises to enhance the host’s amino acid acquisition and inhibit host translation of the proteins include Lgt1-3, SidL, SidL, LegK4, and RavX\textsuperscript{55}. Although the role of translation elongation that resulted out of SidL binding to eEF1A and eEF1B\textsubscript{α} is poorly
understood, this binding is not fully sufficient for impairing the translation. While the mechanisms of RavX, SidI, and SidL remain unclear and require further investigations, it is known that the host’s polypeptide elongation process is inhibited by Lgt1-357. LegK4 can further induce phosphorylation of the host’s Hsp90 by reducing the host’s polypeptide refolding. Furthermore, \textit{L. pneumophila} highly up-regulates the gene synthesis of amino acids, leading to bacterial intracellular growth. LCV-associated bacterial factors play a crucial role in the metabolic pathway of \textit{L. pneumophila}. Additionally, Dot/Icm T4SS and Lsp type II secretion systems (T2SS) are essential for both intracellular and extracellular metabolism. The secreting effectors of T2SS plays a crucial role in \textit{L. pneumophila} infection; more than 25 effector proteins are translocated by T2SS. This system has been associated with LCV membrane in host cytosol. Thus, T2SS enhancing bacterial persistence in human lungs indicates its role in pathogenesis.

**Epidemiological features of \textit{L. pneumophila}**

LD is considered a significant disease, and various countries including the US, Australia, Singapore, Canada, and New Zealand have developed LD surveillance schemes. Nevertheless, globally reported LD data remain rare, contributing to under-recognition, lack of surveillance systems and diagnosis approaches. Resulting in limited data of LD incidence and other related disease-frequency measures. Globally, case distributions are similar regarding both age and sex among countries. It has been shown that the disease is most common among elderly men, while it is uncommon among children. The exact global incidence of LD is still unknown due to the lack of occurrence rates for detecting the disease. However, the US data shows an increase in LD crude incidence in the 21st century. Between 2000 and 2009, the incidence rate has increased from 3.9 per million to 11.5 per million from. This data indicated a seasonal variation, in which approximately 63% of cases occurred in the summer and fall seasons. Incidence was also associated with travel history; almost 25% of patients contracted the illness while travelling. According to the CDC, nearly 10,000 cases of LD were reported by US health departments in 2018 (CDC, 2018). A recent study in 2021, estimated that the LD cases true number is potentially two to three times higher than what is reported. In consideration of the number of travel-associated cases, including those involving, hotel accommodations and cruise ships, effective disease surveillance systems have been created to collect, monitor, and manage data to assess public health actions by identifying sources and trends of infection.

**Clinical outcomes**

LD is atypical pneumonia; it may cause life-threatening respiratory disease, with severe to fatal infection in some cases. Clinically, LD may resemble pneumococcal pneumonia. Although some studies have indicated a distinct clinical syndrome, others suggested that LD and pneumococcal pneumonia share the same clinical and radiographic presentation. Extrapulmonary and pneumonic complications including gastrointestinal and neurological signs are common in patients with community-acquired LD. Symptomatic infection may occur outside the lung due to bacteraemia. The two manifestations of \textit{L. pneumophila} are LD and Pontiac fever. The severity of LD ranges from mild to severe, and more severe pneumonia may require hospital admission. LD has an incubation period between 7 and 14 days, symptoms begin 3 to 14 days after being the exposure. Symptoms include headache, shortness of breath, myalgia, cough, asthenia, and diarrhea. Pontiac fever is characterised by a shorter incubation period than LD; in many cases, it develops within two days. The illness is further considered a self-limited disease and can be asymptomatic. A recent review summarised 136 outbreaks of LD and Pontiac fever between 2006 and 2017. With over 3,500 total cases, 115 outbreaks were LD, while only 4 were Pontiac fever. 17 outbreaks were mixed LD and Pontiac fever. However, interpretation of Pontiac fever is limited due to the lacking an agreed-upon case definition by the scientific scholarly community for either probable or confirmed cases. The infection outcomes depend on bacterial virulence factors such as T4SS together with host immunity. Consequently, the elderly and individuals with chronic lung illnesses such as asthma are at higher risk of developing severe pneumonia. Severe hypoxemia and acute lung injury are also major clinical features of \textit{L. pneumophila}.
induced pneumonia. Furthermore, the serum of patients with *L. pneumophila* has shown high concentrations of inflammatory cytokines, including interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α), granulocyte-colony stimulating factor (GCSF), interleukin-12 (IL-12), IL-6, and IL-8, while IL-10 and IL-4 present with low or undetectable levels.

**Risk factors**

Susceptibility to LD is associated with various host risk factors including smoking, advanced age, chronic cardiovascular, respiratory disease, receipt of a transplant, immune system compromise, diabetes, and alcohol abuse. Also, at increased risk are malignant cancer and chemotherapy patients, including patients with hairy cell leukaemia, haematological malignancies, and solid tumours. In addition, several reports have indicated infection in premature neonates and children. Equally important are the risk factors related to the surrounding environment. Environmental risk factors associated with legionellosis outbreaks include travel, residency in particular facilities such as health care facilities, and poorly disinfected cooling towers. Several recent studies have shown that LD follows seasonal patterns, with the most common activity in summer to early autumn. These patterns are associated with environmental conditions including rainfall changes, climate, humidity, and temperature. Furthermore, nutrients are considered an essential ecological factor that facilitates the biofilm formation of *L. pneumophila*. Many outbreaks have been connected with artificial environments that contain water at high temperatures. In particular, LD most often connected to air conditioning systems, cooling towers, and evaporative condensers.

Consequently, human-made aquatic reservoirs hold the potential to increase human susceptibility to *Legionella*, explaining the rapid increase in *Legionella* incidence in the latter half of the 20th century. Additionally, incidence of the infection may increase during and after the COVID-19 pandemic.

**Antibiotic resistance of *L. pneumophila***

Antimicrobial resistance is a global challenge associated with morbidity and mortality. Although antibiotic resistance is unusual and not yet a major concern in treating *L. pneumophila*, it has been reported in several cases. A recent case of a patient with LD in the Netherlands presented an isolated fluoroquinolone (ciprofloxacin) resistance to *L. pneumophila*. Also, antibiotic resistance in *L. pneumophila* has been identified in several countries such as China. In a study by Rahimi and Vesal, the highest resistance was against ciprofloxacin, erythromycin, clarithromycin, and moxifloxacin with resistance prevalence of 80%, 78%, 52%, and 48%, respectively. The lowest resistance was against rifampicin, doxycycline, and azithromycin with resistance prevalence of 19%, 22% and 26%, respectively. Among macrolides antibiotics, clarithromycin shows high activity compared to azithromycin. In a recent study, minimum inhibitory concentrations were varied between *L. pneumophila* serogroup one and two, making levofloxacin more effective than either minocycline or doxycycline. However, monotherapy involving erythromycin, ciprofloxacin, or rifampicin is not recommended due to rapid antibiotic resistance. While erythromycin was the first choice for treating *Legionella* until the 1990s, it fell out of favour due to the side effects associated with intravenous delivery of the antibiotic. Furthermore, several epidemiological studies have shown that strains of *L. pneumophila* have high resistance against the most common antibiotics, including, ceftriaxone, clarithromycin, rifampicin, tigecycline, azithromycin, erythromycin, moxifloxacin, ciprofloxacin, and doxycycline.

Overall, regulatory treatment of *L. pneumophila* with levofloxacin and azithromycin has proven most effective in reducing transmission of *L. pneumophila*, and is recommended to treat both non- and immunocompromised individuals.

However, fluoroquinolones and macrolides achieve intracellular results therapeutic within tissue and particularly in macrophages, where the bacteria reside. A low concentration of either macrolides or fluoroquinolones can inhibit various *Legionella* strains. Even though the prevalence of fluoroquinolone resistance may be underestimated, highlighting the importance of early *Legionella* infection diagnosis is crucial to ensure timely and accurate antibiotic treatment.

Digital PCR assay has proven helpful as a diagnostic tool to assess antibiotic therapy’s effectiveness. Furthermore, PCR approach to detecting fluoroquinolone-resistant mutations of *Legionella* was implemented.
in 2017\textsuperscript{106}. Given the infrequency of recorded cases of resistance, the Infectious Diseases Society of America recommends either fluoroquinolones or macrolides as antimicrobial therapy\textsuperscript{107}. In addition, a systematic review in 2021 have found no significant difference between fluoroquinolones and macrolides in term of effectiveness in decreasing mortality rate of patients with LD\textsuperscript{107}.

L. pneumophila increased risk during COVID-19

Limiting the growth of Legionella by first preventing L. pneumophila in building water systems is a potential preventive measure. If water is left in a system for more than a week without exchange or flow (e.g. water stagnation), the chance of bacterial growth will be increased\textsuperscript{108}. The easing of COVID-19 lockdown restrictions and global reopening, along with under-recognition of the dangers of Legionella, pose a potential increased risk of developing LD\textsuperscript{108}. Water temperature changes also provide a favourable environment for the bacteria to maintain growth. A recent study, a case of Legionella pneumonia is directly linked with a restaurant’s dishwasher shortly after the SARS-CoV-2 outbreak was brought under control. That emphasised the urgent need of thoroughly inspecting the water systems of different facilities before reopening following closure. Legionella infections are among the respiratory infections that have been diagnosed following lockdown due to the COVID-19 pandemic\textsuperscript{109}. Patients have also been diagnosed with Legionella and COVID-19 co-infection, which can be lethal if left untreated. As a result, the emerging COVID-19 pandemic, warnings of co-infection with other respiratory pathogens are on the rise all over the world. Legionella thrives in poorly treated building water supplies, and outbreaks of LD have been recorded more often in hotels, long-term care centres, and hospitals. COVID-19 infections may increase co-infections risk of Legionella patients that associated with infections waves, posing a significant risk to high-risk COVID-19 patients following the pandemic’s peak and possibly raising disease incidence and mortality. Legionella cases and outbreaks are likely to be an increasingly important public health concern compared to the situation before the COVID-19 pandemic\textsuperscript{109}.

Immune responses to Legionella infection

The immune system has developed different defensive mechanisms against intracellular pathogens, including L. pneumophila. Entry of the bacteria will result in the inflammatory response and activation of immune cells, including macrophages, B lymphocytes, and sometimes natural killer (NK) cells. Consequently, the innate immune response inhibits bacterial growth, mainly in macrophages\textsuperscript{110}. Released IFN-\textgreek{a} via macrophages activates NK and T cells, which increase macrophage resistance against infection. Other released cytokines, such as tumour necrosis factor-alpha (TNF-\textgreek{a}), increase macrophage bactericidal activity and enhance resistance against the disease\textsuperscript{111}. The regulated production of pro-inflammatory cytokines has helped to clear L. pneumonia infection in vivo through the innate immune system\textsuperscript{112}. Also, an accumulation of immune cells during the inflammatory phase of L. pneumophila was observed, including monocytes, dendritic cells (DCs), neutrophils, and T cells\textsuperscript{113}. Engaging the bacterial pathogen with the host led to a disruption of the host’s autoimmune defence mechanisms. In several cases, various cellular processes have been hijacked at the protein level by effector proteins, such as the hijacking of the host cytoplasmic, glycerol kinase enzyme by L. pneumophila to facilitate its metabolic processes\textsuperscript{114}.

Moreover, effector proteins hijack different cellular functions to support bacterial intracellular replication. Accordingly, these effectors can bind, mimic, and modify the host’s proteins, including regulatory elements, enzymes, or transcription factors. Furthermore, the pathogen’s survival within the host cell depends on the formation of LCV to maintain replication\textsuperscript{51}. LCV depends on effector proteins to enter the host cell and survive. Effectors alone have various structures, which raises questions about phenotypes relating to functions. Legionella inside the host cell can modulate the host signalling pathways through the secretion of effector proteins. The effectors are secreted through the secretion system, primarily a membrane complex called the Type IV secretion system (T4SS), which will be discussed in virulence factors section. There is also a correlation between the intensity of cytokine responses and patient severity\textsuperscript{115}. The pro-inflammatory cytokines, such as TNF-\textgreek{a} in autoimmune patients, indicate susceptibility to acquiring LD\textsuperscript{116}. Consequently, TNF-\textgreek{a} plays a crucial role in L. pneumophila induced pneumonia.
pathogenesis. In addition to retrospective analysis, it has been shown that non-LD patients released a higher concentration of IFN-α in response to bacterial lipopolysaccharides (LPS) than patients with LD. These results suggested that low IFN-α levels may be associated with bacterial infection susceptibility. Several in vitro studies supported the role of Th-1 cytokines production via macrophage cells playing a role in restricting bacterial replication. A strong inflammatory response is essential for limiting LD infection in the interaction between L. pneumophila and the adaptive immune response.

Conversely, T and B cells play a critical role in clearing existing infection. T cells become activated after presentation of the bacterial antigens through antigen-presenting cells (APC). APC will uptake the antigen and process it into small peptides, then load it on their major histocompatibility complex to identify T cell receptors (TCR). The most professional APC is DC which has been proven to initiate a specific immune response against L. pneumophila in mice. Notably, in vitro study of bone marrow-derived dendritic cells (BMDCs) infected with L. pneumophila demonstrated that BMDCs induce the production of IFN-α by CD4+ T cells. Simultaneously, the activation of CD4+ T cells is associated with LCV.

**Virulence factors**

Several studies have identified the virulence factors of L. pneumophila. They are associated with pathogenic strains, and are necessary to complete the intracellular infection cycle. Factors related to the L. pneumophila surface structure enhance pathogenesis and promote several processes – for instance, attachment to host cells and intracellular replication (see Table 1.). Those factors include an outer membrane protein (prion), type IV pili, LPS, flagella, T2SS, and PilY1 protein.

**Type IV secretion system**

T4SS is considered major factor of the virulence factors of L. pneumophila; it is a complex protein nanomachine that bacteria utilise to promote proteins and DNA substrates into host cells. The two phyllogenetic types of T4SS are IVA and IVB. The latter is represented by L. pneumophila Dot/Icm T4SS with over twenty proteins and encoded by 27 genes of the Dot/Icm. It includes essential proteins such as DotA, which plays a crucial role in T4SS assembly and activity. In addition, T4SS delivers more than 300 genetic and effector proteins of the bacteria to the host cell’s cytosol.

In a functional T4SS, bacteria can manipulate the trafficking of the host membrane, which allows them to escape phagolysosome fusion and facilitate bacterial replication. This can be implemented through remodelling the LCV into a rough ER-derived organelle. Conversely, bacteria with a deficient stain of Dot/Icm T4SS, such as ∆dotA, cannot replicate intracellularly, because ∆dotA are fused with lysosomes and degraded after they traffic to the endocytic pathway.

T4SS effectors facilitate the intracellular replication of the bacteria by targeting the alveolar macrophages in the lung, injecting neutrophils,

| VF     | Role                                                                 | References |
|--------|----------------------------------------------------------------------|------------|
| EnhC   | Promote intracellular growth by inhibiting the host’s innate immune response through reducing Nod1 and ensuring an efficient replication within macrophages through binding to L. pneumophila Slt. | [134]       |
| Lcl    | Facilitate invasion and cytokines expression.                         | [135]       |
| Hsp60  | Facilitate L. pneumophila entry, phagocytosis, and LCV development.   | [24]        |
| type IV pili | Facilitate adherence to host tissue, biofilm formation, and bacterial survival; promote horizontal gene transfer and enhance the bacterial adaptation to environment. | [52]       |
| LpnE   | Influence trafficking of the L. pneumophila vacuole.                  | [47, 136]  |
| RtxA   | Promote attachment and entry of host cells.                          | [46]        |
| LadC   | Promote attachment to macrophages.                                   | [137]       |

VF: Virulence factor.
and harbouring live bacteria\textsuperscript{130, 131}. Although \textit{L. pneumophila} employs T4SS to inject effector proteins in macrophages, the type IV coupling complex (T4CC) is crucial for delivering the effector proteins to the T4SS\textsuperscript{20}. Notably, the dot/icm DotL, including DotM and DotN, form T4CC\textsuperscript{132}. Different types of effector proteins can be employed through T4CC binding sites. There are two main effector proteins, IcmS and IcmW, IcmSW dependent-effector and IcmSW independent-effector. The latter binds to the T4CC by DotL C-terminal sequence\textsuperscript{20, 133}.

\textbf{PilY1}

Pathogen attachment and entry are crucial to facilitating the pathogen’s modulation. \textit{L. pneumophila} has various adherence determinants that enable entry into host cells, such as surface-associated hsp60, type IV pilin gene\textsuperscript{20}, and the \textit{RtxA} gene\textsuperscript{46} (see Table 1.). One of the more recently described virulence factors of \textit{L. pneumophila} is PilY1, which shares homology with other pathogens such as the PilY1 C-terminal domain of \textit{Pseudomonas aeruginosa} and the PilC1/2 of \textit{Neisseria meningitidis}, and \textit{Kingella kingae}\textsuperscript{138-140}. Accordingly, PilY1 is a cell surface protein that contributes to various virulence features, including biofilm formation and twitching motility\textsuperscript{141}. A study conducted in 2017 showed that the deletion of PilY1 decreased the adhesion of both THP-1 macrophages and A549 epithelial cells to \textit{L. pneumophila}. Simultaneously, reducing the replication rate in THP-1 macrophages, facilitate bacterial survival and replication\textsuperscript{142}.

\textbf{Effector proteins and effector-triggered immunity}

The initial process of recognition and elimination of pathogens results from the engagement between pathogen-associated molecular patterns (PAMPs) and the pathogen recognition receptors (PRRs) of the host. Toll-like receptors (TLRs) consider PRRs located on either plasma membrane or endosomal membrane playing an essential role in initiating an innate immune response against pathogens by recognising PAMPs\textsuperscript{143}. As a result, pro-inflammatory cytokines are released to control the infection\textsuperscript{111}. Nevertheless, bacterial pathogens have developed various virulence factors to avoid immune responses and increase their survival by acquiring the host’s nutrients\textsuperscript{144}. The injection of the bacterial effectors into the host cell is done by highly-specialised secretion systems. Bacterial effectors are utilised by both intracellular and extracellular pathogens, highlighting the importance of these effector proteins in bacterial survival\textsuperscript{145}. A process known as effector-triggered immunity (ETI) was first described in the immune response to plants’ pathogens\textsuperscript{46}. ETI provides detection of the bacterial effector in many multicellular eukaryotes\textsuperscript{147}. In plants, the ETI

\begin{figure}[h]
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\includegraphics[width=\textwidth]{image.png}
\caption{Electron micrograph of \textit{Legionella pneumophila}. A morphology of virulent \textit{L. pneumophila} cell with multiple intracellular inclusions. (Magnification was X 9,000).}
\end{figure}
detects either the effectors or their intracellular activity, while in metazoans it detects only the intracellular effector activity\textsuperscript{147}. In animals, the mechanism of the effectors is indirectly detected by cell-autonomous sensing of effectors’ homeostatic perturbations including pore formation\textsuperscript{148}.

All \textit{L. pneumophila} strain encodes a special group of more than 300 effectors. Thus, the overall number of Legionella effectors to be investigated is override 300. The most studied strains are Philadelphia and Paris, each secreting roughly 330 effectors. Moreover, \textit{L. pneumophila} has been described with more than 25 proteins released by T2SS and various secreted effectors by the Dot/Icm T4SS that exceed 300. One of the unique pathogenicity features of \textit{L. pneumophila} is the total of 350 secreted proteins by \textit{L. pneumophila} that does not correspond to any bacterial pathogen. This is due to the significant number of over 3,000 protein-coding genes per protein with a genome size of 3.2 Mb\textsuperscript{149}. Furthermore, as a result of the protein-coding genes and the effector proteins, \textit{Coxiella burnetti} is considered the closest bacterial pathogen to \textit{L. pneumophila}. The pathogen has a genome size of 2Mb and more than 100 effector proteins, and 2,100 protein-coding genes\textsuperscript{30}.

Although effectors are essential for facilitating bacterial survival and growth, those effectors can paradoxically limit the bacteria by amplifying the production of the pro-inflammatory cytokines in macrophages. As a result, \textit{L. pneumophila} is considered a useful pathogen model for understanding better effector-mediated immunity’s different mechanisms in detecting and eliminating the infection. The collective activity of the effectors leads to an increase in the inflammatory immune response against \textit{L. pneumophila}\textsuperscript{77}. For instance, Lgt1-3, Sidl, and SidL effectors have been associated with activating IL-1\textalpha in macrophages\textsuperscript{150}. Consequently, the selective upregulation of IL-1\textalpha results in an enhanced pro-inflammatory immune response and is considered significant in fighting \textit{L. pneumophila}\textsuperscript{112, 151}. The transitional inhibition which results from metabolic programming is facilitated by the effector-independent mechanism\textsuperscript{21, 152}.

Nevertheless, the host’s amino acid acquisition by \textit{L. pneumophila} is due to effector-independent inhibition of host translation\textsuperscript{153}. Transitional inhibition with pro-inflammatory response in the accidental host represents an example of a conical ETI\textsuperscript{22}. Although some bacterial products, such as effectors, modulate unity, further studies are needed to investigate whether or not harnessing effectors can fight infectious diseases. One example of vaccine adjuvant, the TLR-9 agonist, CpG oligodeoxynucleotides (ODN), which has been used to amplify immune response against parasitic, bacterial, and viral pathogens, including the most recent SARS-CoV-2, the causative agent of COVID-19\textsuperscript{154, 155}. Furthermore, effectors that inhibit immunity have attracted attention as potential drug tools against inflammatory disease\textsuperscript{156}. Since effectors require entry to the cytosol to be fully functional, the recent therapeutically uses of effectors have been implemented in 2017, through fusion to cell-penetrating peptides\textsuperscript{157}.

**Detection and treatment**

Microbial diseases are a real threat and considered one of the leading causes of death globally, particularly in developing countries, shedding light on the importance of accurate detection and identification of microbes. Time to detection is distinctly essential for LD patient outcomes, especially for at-risk individuals. Several detection methods for \textit{Legionella} infection can be implemented via tissue, blood, or respiratory secretions such as sputum. Other methods using urine samples have also been established\textsuperscript{158}. The most common methods for identifying the bacteria include microscopic and cultural techniques and serological tests\textsuperscript{159}. Nevertheless, these more traditional methods have many limitations, encouraging the development of molecular strategies and the invention of Polymerase Chain Reaction (PCR) (Eklund, 2017). Biosensors are the cutting-edge technique in detecting microbial compounds such as proteins, enzymes, and DNA\textsuperscript{77}. Furthermore, methods including urinary antigen tests and nucleic acid amplification testing have also been widely used\textsuperscript{158}. Serology has been effective for historical epidemiological studies even where the infectious agent cannot be isolated despite clear evidence of LD\textsuperscript{5}. However, one main limitation to serology is the false-positive occurrence that may result because of cross-reaction. As alternatives to serologic testing, the urinary antigen test and PCR-based detection methods are considered faster and more user-friendly.

As for pneumococcal pneumonia, the
urinary antigen test has been used to detect the L. pneumophila serogroup one in particular has demonstrated a sensitivity of 70–100% and specificity of 95–100%\textsuperscript{71}. The urinary antigen test also has the advantages of being inexpensive, straightforward, and rapid, making it a first-line screening tool\textsuperscript{81, 160}. However, PCR, with the ability to detect a single pathogenic bacterium, is the most commonly used method. As a result of PCR sensitivity, false-positive results are less likely to occur compared to other methods. Additional advantages of PCR, include speed, high sensitivity, specificity, and accuracy owing to its ability to detect a small amount of nucleic acid\textsuperscript{35}. Of course, L. pneumophila serogroup testing allows detection, however, improvements in assays identifying different serogroups and different Legionella species are required. PCR-based methods have become more commonly used in reference centres, such as L. pneumophila serogroup one detection centres. The development of a fast and accurate multiplexed real-time PCR assay can support other diagnostic methods\textsuperscript{161}. Since L. pneumophila is considered a fastidious bacterium that grows and only slowly with complex nutrients, it is easily identified using biosensors. Such biosensors are a low-cost technique characterised by high specificity and sensitivity. A recent investigation of quantification biosensing of L. pneumophila, has shown that bioassay is an alternative conventional method for L. pneumophila detection\textsuperscript{161}.

CONCLUSION

Many water systems of closed buildings such as educational and business institutions, will have experienced water stagnation, providing a favourable environment for the growth of many bacteria including L. pneumophila. The intracellular L. pneumophila can exploit amoebae and also infect human macrophages. L. pneumophila is the causative agent of LD, a severe and potentially fatal form of pneumonia contracted by inhaling aerosols. L. pneumophila has developed complicated mechanisms to overcome environmental challenges and begin replicating within various niches, increasing its survival in the external environment. A complex regulatory network directs the shift between the two phases of non-virulent and virulent replication. This requires an engagement of both transcriptional and non-transcriptional regulatory elements to assure the effectiveness of the infection cycle. The metabolic changes trigger the morphological stress response, which results in nutrients availability in the surrounding environments. For example, the bacterial multiplication within LCV is supported by serine availability, which is used as a carbon and energy source and leads to increased metabolic activity. In addition, the stringent response of L. pneumophila that facilitates its survival under stress conditions in amino acid depletion. Under stress and starvation conditions, the bacteria enable the expression of virulence genes and shift the overall metabolism to use alternative carbon sources such as glucose. Thus, if these conditions last longer, L. pneumophila is ready to escape the host cell to start a new infection. There remains a serious lack of transmissive phase comprehensive analysis in vivo. Filling such gaps will provide insights into the usage of carbohydrates and crosstalk among the virulence regulatory elements. Urinary antigen tests and molecular methods are commonly used to diagnose infection. However, there are several advantages to effector-based therapeutic techniques in comparison to conventional biologics. Including high specificity, low concentration efficacy, cost-effectiveness, and autonomous translocation. To conclude, through investigation of water stagnation and an understanding of its role in the proliferation of Legionella is required along with lifting restrictions of COVID-19.

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