

Legionella Pathogenesis and Virulence Factors

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Abstract

Legionella is the causative agent of legionellosis or Legionnaires' disease. Infection with Legionella spp. is an important cause of community and hospital-acquired pneumonia. Infection with Legionella is also one of the most common causes of severe pneumonia in community settings, and Legionella is isolated in 40% of hospital-acquired pneumonia cases. Intense research on Legionella pneumophila (*L. pneumophila*) and other Legionella spp. over the last two decades provided insights into the evolution, ecology, epidemiology, and pathogenesis of this opportunistic pathogenic and facultative intracellular bacterium. An in-depth understanding of the ecology and virulence factors of Legionella spp. will contribute to an in-depth comprehension of its pathogenesis and efficient, sustainable elimination of the bacteria from anthropogenic water systems and might lead to the development of novel therapeutics. This review summarizes available information regarding the cellular microbiology, pathogenesis and virulence factors of Legionella spp. with an emphasis on the potential virulence factors and the contribution of these factors to the pathogenesis.

Key words: Legionella, *Legionella pneumophila*, Virulence factors, Pathogenesis

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Introduction

Legionella is an intracellular pathogen and the causative agent of Legionnaires' disease: a severe pneumonia-like disease in which the bacteria infect and replicate in human alveolar and can occur in epidemics of several hundred cases [1]. Accordingly, the severe and atypical pneumonia with a high mortality rate of 15%, was termed "Legionnaires' disease", which appears as a mild respiratory illness and can develop into an acute life-threatening pneumonia or Pontiac fever [2].

The major causative agent of Legionnaires' disease, the *L. pneumophila*, was identified 39 years ago which caused the epidemic of pneumonia that spread during a convent of the American Legion in Philadelphia, USA [3]. After its identification, *L. pneumophila* was shortly characterized as a ubiquitous bacterium that parasitizes free-living environmental protozoa [4]. This finding paved the way for the concept that the ecology and pathogenesis of *L. pneumophila* are closely linked. Fifty-eight Legionella species have been identified to date, and approximately half of these species are linked to Legionnaires' disease. However, with distinct geographical incidence patterns, *L. pneumophila* (serogroup 1) is responsible for the vast majority of clinical cases, which occupies about 90% [2]. It is also demonstrated that only immunocompromised and elderly

person have a high risk to develop a severe disease with respiratory failure. In healthy persons, the innate immune response allows to control *L. pneumophila* infection and the patients are able to effectively eliminate the infection [5]. Moreover, there is no case of transmission from humans to humans reported, indicating that *L. pneumophila* is not completely adapted to infect humans [5]. When Legionella is taken by free-living amoeba or lung alveolar macrophages, complex pathogen-host interactions contribute to its intracellular replication within a sophisticated Legionella-containing vacuole (LCV) [6]. Free-living amoeba in aquatic environments is the natural reservoir and shelter for Legionella [7]. The aerosolized systems such as air conditioning systems and cooling towers are the direct cause for Legionella infection because they make *L. pneumophila* encounter and infect human alveolar macrophages when Legionella-containing water droplets are inhaled [7]. Besides amoeba and macrophages, Legionella can also infect and replicate within ciliate protozoa and mammalian cells like epithelial cells [7]. At cellular level, there has been many similarities in the *L. pneumophila* infection cycle between amoeba and macrophages: both hosts engulf *L. pneumophila* by phagocytosis and the LCV is rapidly formed within the host cytoplasm, avoiding fusion with lysosomes; the establishment of the LCV in both hosts requires the remodeling of the LCV surface by recruiting endoplasmic reticulum (ER) vesicles, ribosomes

and mitochondria [6], therefore creating a “fitness and safe” niche for intracellular replication. These similarities between infection cycle within amoeba and macrophages and the lack of transmission between humans are likely to reveal an evidence that the interaction of *L. pneumophila* with amoeba provides some selective pressure to supply the bacteria with the factors allowing bacteria successful replication within accidentally encountering mammalian macrophages [8,9]. In this respect, environmental amoeba are thought to act as a “trainer”, and *L. pneumophila* has acquired some capacity related to amoeba, to replicate intracellularly also in mammalian macrophages as both free-living amoeba and human macrophages are eukaryotic cells that share conserved molecular pathways targeted by *L. pneumophila* [8-11].

Many studies demonstrated that *L. pneumophila* includes several virulence factors that involve in the whole infection cycle. A number of virulence factors which are encoded by distinct regions of DNA present in the genome of pathogenic bacteria and absent in nonpathogenic strains of the same or related species, named the pathogenicity island locus (PAIs), may be associated with pathogenicity of Legionella, have been well-characterized and reviewed [12,13]. Several studies have shown that Legionella pathogenesis was associated with these virulence factors, because they are required for the whole infection process, including bacterial cell attachment to host cells, survival, intracellular replication, and cell-to-cell spread.

Several studies have discovered many putative virulence genes of Legionella [14-17]. These virulence genes have been described to be the major factors that affecting the ability of Legionella to grow and survive within blood monocytes and alveolar macrophages or within free-living amoebae [13]. This article covers the summarize of different types of virulence factors or genes in Legionella spp. and may help better understanding the appearance of *L. pneumophila* in human communities and lead to new insights on the pathogenesis or virulence strategies of *L. pneumophila* when infecting human macrophages.

Legionella virulence factors and pathogenesis

The primary feature of the pathogenesis of Legionella is their ability to multiply intracellularly. But the whole infection process in both protozoa and mammalian cells including bacterial cell attachment to host cells, survival, intracellular replication, and cell-to-cell spread all indicate its pathogenesis [18]. Studies of the infectious cycles of Legionella are primarily based on microscopic observation by transmission and scanning electron microscopy, and fluorescent microscopy after labeling various bacterial and host cell components [19]. Many studies have determined the process of the bacteria entering the host cells. To date, it is sure that the process is as follows: by coiling phagocytosis, once phagocytized, the Legionella bacteria reside within a unique phagosome and do not fuse with lysosomes or become highly acidic [19]. Previous molecular studies identified several virulence factors encoded by several PAIs and virulence genes. These genes includes the genes in 65 kb PAI, such as dotB, dotF/icmG, dotO/icmB, icmX/proA genes, CpxR and cpxA in cpxR/A PAI,

iraAB PAI, rtxA, lvh PAI and lvgA genes. These genes are thought to be associated with Legionella pathogenesis [20-27], because they act as part of Legionella secretion systems or play important roles in the whole infection process. Many studies demonstrated the relationship between putative virulence factors or genes and pathogenesis using the specific effector mutant Legionella strains to study its ability of replication in human macrophages [28-30]. The direct evidence of these factors or genes participate in Legionella pathogenesis can be revealed by our unpublished data from a study, in which we investigated the distribution of 10 virulence genes including iraA, iraB, lvrA, lvhB, lvhD, cpxR, cpxA, dotA, icmC and icmD in different types of Legionella strains: the distribution frequencies of these genes in reference and environmental *L. pneumophila* strains which considered to be more virulent, were much higher than those in reference non- *L. pneumophila* and environmental non- *L. pneumophila* strains respectively. Furthermore, *L. pneumophila* clinical strains maintained all of these virulence genes compared to other types of Legionella strains. Interestingly, a significant increase of distribution frequencies of these genes was observed in environmental non- *L. pneumophila* strains compared to reference non- *L. pneumophila* strains in our study. These different distribution patterns between reference and environmental non- *L. pneumophila* strains may also reveal that non- *L. pneumophila* strains in environmental water samples can acquire more virulence genes or factors due to the selective pressure as it can also survive in free-living amoeba [31]. These results also highlight the importance of amoeba as “training ground” in Legionella virulence evolution [8].

Virulence factors that related to Legionella cell envelope

Legionella possesses many of the traditional bacterial determinants that are important for pathogenicity in other bacteria, including lipopolysaccharide (LPS), flagella, pili, a type II secretion system (T2SS), and some outer membrane proteins. Many of them constitute *L. pneumophila* cell envelope, including outer membrane vesicles (OMVs), peptidoglycan-associated lipoprotein (PAL), major cell-associated phospholipase A/lysophospholipase A (PlaB), major secreted phospholipase (PlaA), PlaC, major outer membrane protein (MOMP), macrophage infectivity potentiator (Mip), Hsp60 and FeoB (Figure 1) [32].

The OMVs

The outer membrane is the distinguishing feature of all gram-negative bacteria. It is a lipid bilayer composed of phospholipids, lipoproteins, LPS, and proteins. Importantly, the outer membrane is the location of mature LPS molecules and the shedding of OMVs [32]. Similar to most of gram-negative bacteria, Legionella sheds vesicles are from its outer membrane. These OMVs are spherical lipid bilayer structures with a diameter of 100-200 nm, contain outer membrane components, periplasmic proteins, and great number of virulence-associated proteins which display lipolytic and proteolytic activities [33]. OMVs shedding are first observed from *L. pneumophila*. The OMVs shedding can already be found in electron micrographs and this finding is published shortly after the discovery of this bacterium [34]. Later, it is shown that OMVs of *L. pneumophila* are produced both extracellularly and

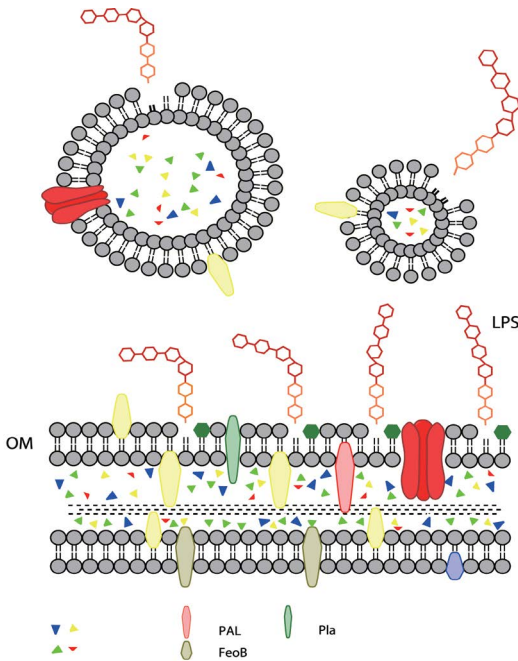


Figure 1 Overview of the Legionella virulence factors that constitute cell envelope. CP: Cytoplasm; IM, Inner Membrane; PP: Peri Plasm; OM: Outer Membrane; OMVs: Outer Membrane Vesicles. They involve in binding with and delivery of packaged materials into the eukaryotic cells; LPS: Lipopolysaccharides, it induces the secretion of proinflammatory cytokines from human monocytic cells; PAL: Peptidoglycan-Associated Lipoprotein, it can induce the secretion of proinflammatory cytokines; FeoB, iron transporter, it plays important roles in Legionella uptake of Fe²⁺; PlaB, phospholipase A/lysophospholipase A, it promotes replication of *L. pneumophila* in the lungs and dissemination to the spleen; MOMP: Major Outer Membrane Protein, it plays an important role in Legionella attachment to host cells; Mip, Macrophage infectivity potentiator, it enables to transigrate across tissue barriers and plays other roles in Legionella infection [32].

within host cells under different growth conditions. Moreover, they have been shown to adhere to and fuse with eukaryotic host cell surfaces [33]. OMVs have been reported to contribute to a key and early event of the Legionella infection cycle because of the action of the OMVs including their models of binding with and delivery of packaged materials into the eukaryotic cells. These packaged materials include virulence protein and some other virulence factors. They can also inhibit the fusion of phagosomes with lysosomes, avoiding the host cell killing of Legionella bacteria [35]. Based on an experiment performed on a human lung tissue explant model, OMVs are demonstrated to have new functions during Legionella infection: OMVs are located in alveolar macrophages, bind predominantly to the surfaces of alveolar macrophages and can be detected in their cytoplasm [36]. Stimulating human lung tissue explants with OMVs resulted in distinct tissue damage with epithelial cell delamination in affected alveoli and damage to collagen structures in septa and connective tissue fibers, starting approximately 24 h after

infection [36]. Therefore, the OMVs act not only binding with and delivery of packaged materials, but also enhance *L. pneumophila* virulence.

The PAL, Pla family and LPS

In addition to OMVs, Mip, PAL, LPS and MOMP are all associated with virulence. As a 19-kDa protein, PAL is a species-common immunodominant antigen for the diagnosis of Legionnaires' disease [37,38]. This protein can activate macrophages via toll-like receptor 2 and induce the secretion of proinflammatory cytokines such as interleukin-6 and tumor necrosis factor-alpha, finally aggravate the inflammation in Legionella infection [38]. The intracellularly replicating *L. pneumophila* consists of an extraordinary variety of phospholipases, including at least 15 different phospholipases A (PLA), in which a cell-associated phospholipase, PlaB, displays contact-dependent hemolytic activity and plays an important role in guinea pig infection [30]. Active PlaB is outer membrane-associated and at least in parts surface-exposed, displays the most prominent PLA activity in *L. pneumophila*. The transport of PlaB to the outer membrane is not dependent on the type I (T1SS), II (T2SS), IVB (T4BSS) or Tat secretion pathways and the activity of PlaB is not dependent on the presence of the Mip or the major secreted zinc metalloproteinase A (MspA). Although PlaB is not essential for replication in protozoa or macrophage cell lines, plaB mutants impair the replication of *L. pneumophila* in the lungs and the dissemination of the bacteria to the spleen in the guinea pig infection model [30]. Infection of mouse macrophages with *L. pneumophila* wild type, plaB knock-out mutant, and plaB complementing or various mutated plaB-harboring strains showed that catalytic activity of PlaB promotes intracellular replication. Further study also demonstrated that PlaB is a virulence factor that assembles in inactive tetramers at micromolar concentrations with oligomer dissociation at nanomolar concentrations activates PLA activity [39]. As like the PlaB, PlaA and PlaC are also important for *L. pneumophila* virulence, although single mutants of which demonstrate no discernible phenotype [40]. These two phospholipases may act to break down lung surfactant in Legionella infection and the phospholipase activity of these factors make it act as signaling molecules, influencing important host functions such as inflammation and apoptosis [41].

LPS are located in the outer leaflet of the outer membrane and they are a major immunodominant antigen of Legionella. Although *L. pneumophila* LPS and its inner core Lipid A does not function as a classical endotoxin, and their potential to induce the secretion of proinflammatory cytokines from human monocytic cells are about 1000 times less than that of Enterobacteriaceae, LPS still consider to be a potential virulence factor [42]. Other virulence factors that related to Legionella cell envelope are MOMP, Mip, Hsp60 and FeoB. Their virulence are associated with cell attachment and entry to host cells or intracellular survive and replication.

Virulence factors that related to Legionella cell attachment to host cells

There is still a lack of knowledge surrounding the issue how the bacteria are internalized by eukaryotic cells and ultimately released to infect new cells. But there is a consensus that the

Legionella cell attachment and entry to host can be considered as the first step and most important step in Legionella infection cycle, with many proteins and genes involve in. Bacterial factors identified till now that participate in the attachment and entry of *L. pneumophila* into host cells include at least five proteins, EnhC, Hsp60, LpnE, RtxA and LvhB2 [43-46]. However, few of these factors have been shown to play a definitive and direct role in bacterial uptake. In addition, MOMP, type 4 pili, LadC, Lcl also act critical part in these processes. The conclusion of the major factors that related to Legionella cell attachment to host cell is shown in **Table 1**.

The EnhC

EnhC is a periplasmic protein and is required for efficient replication in macrophages and for the maintenance of cell wall integrity. Therefore, its contribution to bacterial invasion is indirect, although the definitive role of EnhC is binding to the *L. pneumophila* Slt and interferes with its function, with the consequence of facilitating intracellular growth of *L. pneumophila* by reducing host cell Nod1 innate immune recognition of bacteria [47].

The Hsp60

One of the most abundant proteins synthesized by *L. pneumophila*, particularly during the growth in a variety of eukaryotic host cells, is Hsp60, a member of the GroEL family molecular chaperones. For Legionella, htpB gene encodes this 60-kDa heat shock protein that enhances invasion and cytokine expression in macrophages [48]. The chaperonin Hsp60 is often present at unusual locations, for example, in association with the bacterial cell surface and mediates adherence to host cells [49]. *L. pneumophila* Hsp60 modulates macrophage function through a mechanism that involves surface interactions in the absence of Hsp60 internalization [50], suggesting that surface-exposed Hsp60 play an important role in the pathogenesis of Legionnaires' disease. Hsp60 also contributes to the recruitment of mitochondria to the nascent LCV, as inert beads coated with Hsp60 are associated with mitochondria following invasion [51]. Hsp60 may play a dual role in bacterial entry and the early development of the LCV, suggesting that these two events are related. In this respect, Hsp60 has been observed during the early infection of macrophages by *L. pneumophila* [52].

The MOMP

The MOMP is encoded by mompS gene and plays an important role in Legionella attachment to host cells. Previous study demonstrated that complement receptors CR1 and CR3 on human monocytes mediate phagocytosis of *L. pneumophila* [53]. Futher study illustrated that *L. pneumophila* fixes complement C3 to its surface by an alternative pathway of complement activation and this action was mediated by MOMP, finally lead to more convenient entry of Legionella to host cells [54].

The type IV pili

In addition to Hsp60 and MOMP, type IV pili also involve in Legionella attachment and the entry to host cells. The type IV pili (T4P) are characterized by a conserved hydrophobic amino-terminal domain [55]. Pili have been demonstrated on the surface of Legionella by transmission electron microscopy [55]. The most commonly function of T4P for Legionella is adherence to host tissues, which facilitates the bacteria invasion. The encoding genes of Legionella pili include pilB, pilC, pilD, pilE, pilM, pilN, pilO, pilP and pilQ. They are all involved in adherence and intracellular replication of *L. pneumophila*. Additionally, T4P also participates in biofilm development and formation, promoting Legionella adherence and aggregation in biofilms, remodeling biofilm architecture through twitching motility and facilitates the survive of bacteria cells in variable environmental conditions [56]. Studies of bacterial evolution have revealed the extensive contribution of horizontal gene transfer (HGT) to the shaping of bacterial genomes. HGT probably contributes to the evolution of Legionella and its adaptation to different environments [57-59]. The virulence genes are able to be horizontally transferred from one *L. pneumophila* strains to other Legionella strains in its natural environment [59]. T4P have long been recognized as important systems for the acquisition of exogenous DNA in Legionella, by acting as receptors for transducing bacteriophages and by their involvement in competence and conjugation [60]. Therefore, T4P plays important role in Legionella virulence shaping.

The LpnE

The LpnE and its encoding gene, lpnE was identified to contribute to the virulence of *L. pneumophila* by performed genomic subtractive hybridization between a *L. pneumophila* serogroup 1 strain and a *L. micdadei* strain [46]. The lpnE gene was shown

Table 1 Virulence factors that related to *Legionella* cell attachment and entry to host cells.

Roles	Virulence factors	Encoding genes	Involved functions	References
Cell attachment	EnhC	<i>enhC</i>	maintenance of cell wall integrity, facilitating <i>Legionella</i> intracellular growth	47
	Lcl	<i>htpB</i>	enhances invasion and cytokine expression, recruitment of mitochondria to the nascent LCV	48-51
	Hsp60	<i>mompS</i>	mediate phagocytosis of <i>L. pneumophila</i>	53,54
	MOMP	<i>pilB-E, pilM-Q</i>	adherence and intracellular replication, biofilm development and formation, horizontal gene transfer	56-59
	type IV pili	<i>lpnE</i>	entry of <i>L. pneumophila</i> into macrophage, influenced trafficking of the <i>L. pneumophila</i> vacuole	46,61
	LpnE	<i>rtxA</i>	mediate <i>L. pneumophila</i> attachment to human cells	43,62
	RtxA	<i>lcl</i>	adherence and invasion of host cells	63
	LadC	<i>ladC</i>	adhesion to macrophages	64

to be required for full entry of *L. pneumophila* into macrophage cell line THP-1, and epithelial cell line, A549 cells [46]. After that, LpnE was then demonstrated to be with Sel1 repeats (SLRs). Further studies have shown that LpnE is also required for efficient infection of *Acanthamoeba castellanii* by *L. pneumophila* and for replication of *L. pneumophila* in the lungs of A/J mice [61]. In addition, LpnE's role in host cell invasion is dependent on its SLR regions, The LpnE influenced trafficking of the *L. pneumophila* vacuole, similar to the case for EnhC [61]. LpnE was present in *L. pneumophila* culture supernatants, this fact suggested that the LpnE may interact with a eukaryotic protein. All of these informations highlighted the contribution of LpnE to *L. pneumophila* virulence and the importance of the intrinsic SLR regions to LpnE function.

The RtxA and other factors

RtxA appears to be involved in the attachment and entry of *L. pneumophila* into *Acanthamoeba castellanii* but the detailed mechanism of RtxA function is still unknown [62]. The RtxA have also been shown to mediate *L. pneumophila* attachment to human cells, as rtxA mutants displayed a diminished adherence and entry into human epithelial and monocytic cell lines [43].

A *L. pneumophila* collagen-like protein named Lcl was shown to contribute to the adherence and invasion of host cells. It was demonstrated that the number of repeat units present in Lcl had an influence on these adhesion characteristics [63]. A putative *L. pneumophila*-specific adenylate cyclase, named LadC that is present in the bacterial inner membrane is also identified in adhesion to macrophages [64].

Virulence factors that related to Legionella survive and multiply in host cells

Legionella entombment in amoeba cysts has been observed in previous study [23]. After enters to the host cells, Legionella needs to survive and multiply in different environmental conditions of the host cells. Many factors participate in these processes.

The Mip protein and its role in virulence

The gene encoding the peptidyl-prolyl-cis/trans isomerase (PPIase), Mip, is one of the first genes that proved to be associated with the ability of *L. pneumophila* to replicate in eukaryotic cells, and the mip nucleotide sequence has been used as a target for Legionella molecular diagnostics and typing for about 2 decades [65-67]. This protein is a 24-kDa protein and specific expressed in the surface of Legionella bacteria, where the protein exists as a stable homodimer and in association with OMVs [33,68,69]. Based on its PPIase activity, it can bind to collagen IV and therefore enables *L. pneumophila* to transmigrate across tissue barriers [70]. With this activity, the Mip protein contributes to *L. pneumophila* virulence. However, clearly evidence that proved Mip linking to Legionella pathogenesis is based on studies using mip-positive, mip-negative and mip-mutant *L. pneumophila* strains [71,72]. Further studies have shown that mip mutants of *L. pneumophila* are defective for replication in eukaryotic cells including macrophages, epithelial cells, and amoebae and the replication capability of which is attenuated in both guinea

pig and mouse models [28,73]. The Mip belongs to the enzyme family of FK506-binding proteins (FKBP), can bind to collagen of types I, II, III, V, and VI, and shares amino acid sequence similarity with the eukaryotic family of FK506 binding proteins. As a structural and functional mimic of a eukaryotic protein, the crystal structure of Mip make it convenient integrates with the FK506 protein of macrophage, and then inhibits the activation of this cell [69], and promotes intracellular replication. Mip may also act synergistically with other secreted proteins of *L. pneumophila*, such as a p-nitrophenol phosphorylcholine hydrolase [74]. Additionally, as a strongly basic protein (pI 9.8) with an N-terminal signal sequence, Mip mediates aerobic oxidation and cytoplasmic membrane depolarization of macrophage [75]. This character helps Legionella survive in host cells and resistance to the killing of host cells.

The Sid family

Although SidC, SdcA (a SidC paralogue) and SidJ are all shown to be functional for LCV recruitment of ER-derived vesicles [76,77], SidC and SdcA play minor roles in maintaining LCV membrane integrity because *L. pneumophila* deficient in both factors can replicate as efficiently as the wild-type strain. However, deletion of SidJ can cause a severe growth defect in both macrophage and amoeba. In contrast, its paralogue, SdjA, plays a minor role only in the amoeba [76]. These informations indicate that in these three potential virulence factors, SidJ is of ultimately importance. It is an effector protein which secreted by type IVB secretory system, and is required for efficient recruitment of endoplasmic reticulum proteins to the bacterial phagosome [76].

Virulence Factors that Related to Iron Acquisition

Legionella spp. are fastidious organisms and iron acquisition is required for Legionella growth intracellularly or extracellularly. Many studies have highlighted the importance of iron for *L. pneumophila* virulence and replication by exposing *L. pneumophila* bacteria to iron-limiting environments *in vitro* [78]. Iron is also important during intracellular replication in host cells, as monocytes and macrophages that have been treated with an iron chelator do not support *L. pneumophila* replication [15]. Thus, some factors that related to iron uptake or acquisition are likely to be critical for Legionella virulence. There have been two means for Legionella uptake iron. The first means by which *L. pneumophila* acquires iron is through the Fe³⁺-chelating activity of a siderophore, the most common factor for iron acquisition and promoting lung infection [79-81]. When *L. pneumophila* grows in low-iron conditions or chemically defined medium (CDM), it secretes this low-molecular-weight, non-protein, high-affinity, ferric-iron chelator, siderophore and is also known as legiobactin. Legiobactin is both reactive in the chrome azurol S (CAS) assay and capable of stimulating the growth of iron-starved Legionella [80]. Legiobactin is required for optimal intrapulmonary survival by *L. pneumophila*. Legiobactin secreted by the wild type can aid growth of the mutant in trans, it promotes something other than intracellular infection of resident lung cells. Many but not all other Legionella species appear to produce legiobactin [82]. The LbtA and LbtB proteins and their coded genes, lbtA and lbtB are absolutely required for legiobactin activity and the production

and export of the *L. pneumophila* legiobactin. LbtA has homology to siderophore synthetases, and LbtB is akin to inner membrane siderophore exporters. Thus, cytoplasmic LbtA is likely involved in the synthesis of legiobactin, whereas LbtB promotes the transit of the siderophore across the inner membrane of Legionella prior to its final export. In addition, LbtU, LbtC and Cyc4 also play roles in iron acquisition. Cyc4 promote secretion of the legiobactin, LbtU and LbtC with their coded genes, lbtU and lbtC mediate uptake of Fe³⁺ legiobactin complexes in which LbtU, as a TonB-independent receptor for the siderophore specifically promotes *L. pneumophila*'s utilization of legiobactin and likely acts as the receptor for the Legionella siderophore, LbtC involves in iron regulated [81,83-85].

The second means by which Legionella acquires iron is via the uptake of Fe²⁺, with the inner membrane protein FeoB translocating ferrous iron into the cell's interior [86]. Once the iron-siderophore is internalized across the outer membrane, it is likely acted upon by a periplasmic ferric reductase to yield ferrous iron that is transported across the inner membrane by FeoB [87]. The FeoB is important for extracellular growth and promotes replication in macrophage [86]. The inability to mutate both feoB and lbtA indicates that at least one of these two pathways is essential for *L. pneumophila* viability and virulence [83]. Both of legiobactin and FeoB are required for lung infection by *L. pneumophila* [80,86], indicating that these two ways and associated factors are all important for Legionella virulence.

It is sure that *L. pneumophila* uses both a ferrisiderophore pathway (through Fe³⁺-chelating activity of a siderophore) and ferrous iron transport (uptake of Fe²⁺) to obtain iron. Another way for *L. pneumophila* to obtain iron is found recently. Two molecules secreted by *L. pneumophila*, homogentisic acid (HGA) and its polymerized variant (HGA-melanin, a pyomelanin), are shown to be key components for *L. pneumophila* iron acquisition, because they are able to directly mediate the reduction of various ferric iron salts, including ones that *L. pneumophila* likely encounters in natural aquatic and mammalian host environments [88]. They are also shown to enhance the ability of *L. pneumophila* and other species of Legionella to uptake iron, stimulate *L. pneumophila* growth under low-iron conditions, and are made in inverse fashion to secreted siderophore activity [88].

L. pneumophila also produces a multicopper oxidase, McoL,

which is essential for aerobic extracellular growth under iron-limiting conditions or where ferrous iron is the only iron source [89]. McoL may also help prevent the toxic effects of ferrous iron and act as a virulence factor during aerobic growth.

Besides the factors mentioned above, several other factors also appear to be important for iron acquisition and assimilation, most notably the cytochrome C maturation (ccm) locus that are essential for intracellular multiplication [16,90]. Iron intake or assimilation locus includes iraA and iraB [15]. IraA encodes a protein with 272 amino acid residues which is similar to the sequences of DNA methyltransferase. In contrast, iraB encodes a protein with 501 amino acid residues. These proteins play different roles in Legionella virulence and pathogenicity. Studies in iraA or iraB mutant Legionella strains demonstrated that iraA is important for intracellular replication [14], while iraB contributes to extracellular growth under iron-limiting conditions [15]. The above information is concluded in Table 2.

The secretion systems of the Legionella

Many bacterial pathogens utilize specialized protein secretion systems to deliver virulence effector proteins or other factors into host cells. These virulence factors interfere with the antimicrobial responses of the host and facilitate the survival of the pathogen [91,92]. Legionella also can secrete a very large number of factors that promote virulence and/or intracellular infection of host cells. These virulence factors include both proteins and non-protein molecules that critically promote infection.

As an environmental organism, Legionella has ability to survive and replicate in amoebae and some types of human cells. The Legionella bacterium promotes their uptake by alveolar macrophages and epithelial cells, where they replicate within an intracellular vacuole that avoids fusion with the endocytic pathway [46]. The secretion systems of Legionella bring into play important roles in this process. Gram-negative bacteria often have six, and perhaps eight systems that facilitate secretion from within the bacterium to the extracellular milieu and/or into target host cells; named type I, II, III, etc [93]. Legionella is shown to govern the formation of LCVs and other pathogen-host interactions through distinct protein secretion systems, such as the Dot/Icm type IV secretion system (T4SS) and the Lsp type II secretion system (T2SS). Many other studies also have shown that

Table 2 Virulence factors that related to iron acquisition.

Roles	Virulence factors	Encoding genes	Involved functions	References
uptake of Fe ³⁺ iron	LbtA	<i>lbtA</i>	synthesis of legiobactin	83
	LbtB	<i>lbtB</i>	siderophore export	83
	LbtU	<i>lbtU</i>	utilization of legiobactin	81
	LbtC	<i>lbtC</i>	Iron uptake regulation	85
	Cyc4	<i>cyc</i>	secretion of the legiobactin	85
uptake of Fe ²⁺ iron	FeoB	<i>feoB</i>	siderophore across the inner membrane	86, 87
Other roles	Pyomelanin		enhance the iron uptake	88
	McoL	<i>mcoL</i>	aerobic extracellular growth	89
	Ccm locus	-	intracellular multiplication	16,90
	-	<i>iraA</i>	intracellular replication	14
	-	<i>iraB</i>	extracellular growth	15

the Dot/Icm Type IV secretion system, the Lsp Type II secretion system and the Tat, Lss, Lvh secretion systems are essential for *L. pneumophila*. We describe their roles in *L. pneumophila* virulence and the bacteria interactions with its hosts.

The Dot/Icm Type IV secretion system

T4SS are versatile multi-protein complexes that can transport DNA and proteins to recipient bacteria or host cells [94,95]. Three main T4SS classes have been distinguished: T4SSA, T4SSB, and genomic island-associated T4SS (GI-T4SS), based on structural and organizational similarity [96]. The most important T4SS of the Legionella is the Dot/Icm Type IV secretion system. It belongs to T4SSB. Components of the *L. pneumophila* Dot/Icm T4SS seem to be involved in all aspects of the intracellular biology of *L. pneumophila*, including secreting and translocating multiple bacterial effector proteins into the vacuolar membrane and cytosol of the host cell [2,97]. About 300 different "effector" proteins are translocated into host cells through this system, and many of which have similar characters to eukaryotic proteins or carry eukaryotic motifs [98,99]. They are predicted to allow *L. pneumophila* manipulating host cell processes by special functional mimicry [97]. In these effector proteins, at least 240 of which have carboxyl-terminal signal sequences that allow translocation by the Dot/Icm system [100]. These effectors target a variety of host proteins that control secretory traffic, translation, and cell survival to promote the formation of a replication vacuole [101]. For instance, in macrophages, recruitment of Rab1 and Arf1 to the LCV is mediated by the Dot/Icm-dependent translocated effectors SidM/DrrA and RalF, respectively [102,103]. Other Dot/Icm translocated substrates also participate in the recruitment of ER vesicles, i.e., LidA attaches to the cytoplasmic face of the LCV and synergizes with SidM/DrrA in the recruitment of Rab1 [103]. Recently, a novel zinc metallophospholipase C family protein, named PlcC/CegC1 together with the PlcA and PlcB is shown to promote *L. pneumophila* virulence [104]. The Dot/Icm T4SS is not only required for intracellular replication and the establishment of the LCV, but also involved in bacterial entry, the inhibition of host cell apoptosis, and the egress of *L. pneumophila* from host cells [105,106]. The nomenclature of the Dot/Icm T4SS is from the *icm* locus and the *dot* locus. The *icm* genetic locus first comprises four putative genes, the *icmWXYZ*, are important for Legionella intracellular multiplication [107,108]. In contrast, genetic locus which was designated to be *dot*, could complement the mutant with defective intracellular replication and an inability to establish the ER-derived LCV [109]. This complementing locus includes one open reading frame that encoding a 1048-aminoacid protein, was designated *dotA*, and the split nomenclature for this secretion system has remained [110]. More and more *dot/icm* genes have been defined to be required for Legionella virulence, including *dotH*, *dotI*, and *dotO*. They were identified after initial discoveries of these loci, and are all essential for intracellular growth and the evasion of the endocytic pathway. The *icm* locus, *icmGCDJBF* and *icmTSRQPO* were also identified and are implicated in macrophage cell death [111,112]. The genes of this secretion system are found on two distinct regions in the chromosome of Legionella, each approximately 20 kb in length. Region 1 comprises *dotDCB* and *dotA-icm VWX* [113]. Region 2 includes 18 genes, most are with *dot* and *icm* designations [114]. In addition, the Dot/Icm system is

ancestrally related to DNA conjugation systems and has retained the ability to mobilize certain plasmids [115]. In this aspect, the T4SS are important for the virulence of several pathogens including the Legionella and may transfer nucleic acids, proteins, or complexes of both to recipient cells [116]. Thus, this system may play an important role in horizontal gene transfer and the acquisition of virulence genes or factors.

Recent studies also demonstrate that the Dot/Icm components form a multi-protein apparatus that spans both the inner and outer membranes of the bacterial cell wall [26]. It is clear that the Dot/Icm apparatus forms a translocon that delivers multiple bacterial effector proteins into the host cell in a manner that is functionally analogous to that of a type III secretion system (T3SS). Indeed, similar to this T3SS, the Dot/Icm T4SS is believed to utilize a network of cytoplasmic chaperones, the best characterized of which are *IcmS* and *IcmW*, that bind to effector proteins and facilitate their translocation [117], *IcmR* and *IcmQ* form a stable heterodimer that is localized to the Legionella cytoplasm [118], where *IcmR* may act as a chaperone to prevent aggregation or nonspecific interactions of *IcmQ* with other components [119].

The Lsp Type II Secretion System

In addition to the Dot/Icm T4SS, *L. pneumophila* also possesses a T2SS for Legionella secretion pathway which termed Lsp. This secretion system is required for full virulence and environmental persistence of Legionella, including intracellular growth in amoeba and extracellular survival in low-temperature water samples [120-122]. In gram-negative bacteria including Legionella, T2SS exists as highly conserved protein secretion machines that play an important role in the disease progression of variables bacterial pathogens through the export and directed release of toxins, proteases, and other enzymes [123]. Evidence for this can be partly found in a study in A/J mice. The study demonstrated that Lsp mutants do not increase in numbers in the lung, indicating that Lsp is critical for *L. pneumophila* infection of and pathogenesis in mammalian hosts [92,124]. The Legionella T2SS promotes the export of at least 25 proteins, 17 enzymatic activities, and a surfactant that facilitates surface translocation [125,126]. Some of them have been identified link to infection of amoebae and to optimal lung infection [127,128]. It also has been demonstrated that *L. pneumophila* T2SS, Lsp promotes the intracellular infection of lung epithelial cells, dampens the cytokine secretion from infected macrophages and epithelia, and limits the levels of cytokine transcripts in infected macrophages [129]. The Lsp has 12 components located in five gene locus throughout the chromosome. These components include the prepilin peptidase *pilD*, which processes the pilin of type IV pili and pseudopilins of the T2SS and is definitely required for the virulence of the intracellular Legionella [130]; the outer membrane secretin and ATPase *IspDE*; the pseudopilins *IspFGHIJK*; and *IspC* and *IspLM*, which are predicted to promote secretion, survive and virulence [124,131]. Study on mutant strains of *L. pneumophila* lacking *IspDE*, *IspG*, or *IspK* demonstrate replication defect in macrophages and reduction of capacity to replicate in the lungs of mice [124,132]. These results illustrated that Lsp is important to Legionella virulence.

The ability of Legionella to keep viable under a wide range of

conditions is fundamental to its environmental persistence. The Lsp system also presumably secretes factors that aid *L. pneumophila* persistence at low temperatures, both in tap water at temperatures ranging from 4°C to 17°C and in the presence of amoebae at temperatures of 22°C to 25°C; as the growth of lsp mutants is stimulated by the addition of culture supernatant from the wild-type strain [121]. Other major factors related to Lsp T2SS includes a homologue of the LpxP lipid A acyltransferase; an RNA helicase, CsdA, which was particularly important for bacterial survival; and RNase R, which was implicated previously in replication at low temperatures [122,133].

The Tat, Lss, and Lvh Secretion Systems

In addition to the Dot/Icm and Lsp protein secretion systems, *L. pneumophila* harbors other systems, including a type I secretion system (Lss), a twin-arginine translocation system (Tat), and a second type IV secretion system, belongs to T4SSA class (Legionella vir homologues, Lvh). They play different roles in aiding Legionella virulence. The lssXYZABD locus includes the typical components of a type I secretion system, including an ABC transporter (LssB) and a membrane fusion protein (LssD) [134]. Lss may also play a role in the biology of *L. pneumophila*, although the secretion system appears to be largely dispensable for host-pathogen interactions [134]. In contrast, the Lvh system plays a role in certain aspects of intracellular replication by complementing Dot/Icm function for Lvh region was required to rescue the host cell uptake of Dot/Icm mutants, although it is not required for intracellular bacterial replication in macrophages and amoebae but seems to contribute to infection at lower temperatures and inclusion in *Acanthamoeba castellanii* cysts [27]. These finding indicates that both the Dot/Icm and Lvh systems are conditionally required for certain virulence such as host cell invasion.

The Tat system complements the general secretory pathway by transporting folded proteins across the inner membrane of cytoplasmic, and is critical to virulence in many pathogens. In *L. pneumophila*, the Tat pathway contributes to biofilm formation and intracellular replication and infection in macrophages and amoebae and aids growth under low-iron conditions [135,136]. The phospholipase A of *L. pneumophila* is Tat-dependent, and Legionella tat mutants also displays virulence defect [136]. In addition, Legionella Tat and the encoding gene tatB gene are also shown to facilitate secretion of phospholipase C [136]. All of these finding indicate the importance of Tat to Legionella virulence.

There seems to be little crossover between the Lsp and Tat secretion systems because some of the Lsp substrates seem to utilize the Tat pathway to reach the periplasm, and the supernatants of tatB mutants exhibit normal levels of Lsp-dependent enzymatic activity [136]. Additionally, a mutant strain of *L. pneumophila* lacking both Tat and Lsp systems demonstrates a defect in replication greater than that of either of the single mutants [136]. This result supports the evidence that the Tat and Lsp systems work independently and also indicates the importance of these two systems to Legionella virulence maintenance.

Factors that regulate Legionella virulence

The environmental and intracellular niches of Legionella expose the bacteria to a range of nutrient and temperature conditions that require it to be highly adaptable. As like to other intracellular

pathogens, *L. pneumophila* has a biphasic growth cycle, replicative phase: express few virulence traits, transmissive phase: become highly motile and resistant to various stresses [137]. The transmissive phase of Legionella allows it to egress from spent host cells and start the next round infection, and this process coincides with the expression of many virulence determinants. Thus, the Legionella growth and the virulence can be regulated by many factors due to the phase conversion. The factors that involve in regulation of Legionella growth are also related to virulence *L. pneumophila*.

RpoS and its role in regulation of virulence

RpoS is an alternative sigma factor of RNA polymerase and regulates motility, sodium sensitivity, and evasion of the endocytic pathway, plays a critical role in regulation of transmission and virulence of *L. pneumophila* [138,139]. This protein is important for survival in osmotic shock but not other stress conditions in exponential phase of Legionella [140]. In stationary phase, although cells become more stress resistant, RpoS is dispensable [140]. It is clear that an isogenic mutant of rpoS makes Legionella unable to replicate within *Acanthamoeba castellanii* [140]. Among other related factors, in exponential phase, RpoS directly regulates the expression of flhA, which is of special importance for regulating contact-dependent cytotoxicity, lysosomal avoidance, infectivity, and biofilm formation [29,141]. In exponential phase, RpoS also downregulates the transcription of *L. pneumophila* virulence genes csrA, letE, and flaA and represses bacteria motility, infectivity, and cytotoxicity [138]. RpoS also regulates the transcription of mip gene which is severely impaired in postexponential phase rpoS mutants [142]. Production of phospholipase and lipophospholipase, two virulence factors, are also positive controlled by RpoS [143]. Similar to phospholipase and lipophospholipase, ProA, a secreted virulence protease that is cytotoxic to macrophages and is important for virulence in a guinea pig model, also positively regulated by RpoS [143]. RpoS also regulates the expression of the ankyrin genes that play a critical role in intracellular growth within amoeba and human macrophages, and LqsR-regulated genes that are involved in virulence, motility, and cell division [144,145]. RpoS is also shown to be crucial for the pore-forming activity of *L. pneumophila* and adaptation to phagosomal intracellular environments during infection [139]. Although RpoS only has a minor effect on the expression of the Icm/Dot genes, many genes encoding Icm/Dot secreted proteins require RpoS for full expression [146,147].

Factors that related to the regulation of the flagellum

In *L. pneumophila*, the regulation of the flagellum and the expression of virulence traits are linked. FlhA, FleQ and RpoN are the major regulators of the flagellar regulon [29]. These proteins are proved all necessary for full *in vivo* fitness and involved in the invasion of *L. pneumophila* strains Corby and Paris [148,149]. It is also known that *L. pneumophila* rpoN, fleQ, flhA and flaA mutant strains are non-flagellated and non-motile [29], indicating they are important for Legionella virulence. FlhA is required for the *in vivo* fitness, FleQ and RpoN not only influence the expression of flagellar genes, but they are also involved in the expression

of various known virulence genes of *L. pneumophila* [148]. A recent study in *L. pneumophila* strains Corby and Paris and *Acanthamoeba castellanii* cysts demonstrate that Δ rpoN and Δ fleQ mutant strains are less able to survive in direct competition to the wild-type strain, demonstrating that they are reduced in their virulence or fitness. In contrast, Δ fliA mutant exhibits only a slightly reduced fitness [149].

Two component response regulators

Two-component systems, also called two component response regulators, are widespread signal transduction devices in bacteria that enable them to respond to environmental stimuli mainly via changes in gene expression. These systems are used by many pathogenic bacteria including the *Legionella* to control the expression of their virulence genes [150]. In parallel with RpoS and those flagellar regulon, other two component response regulators contribute to the regulation of transmissive traits. Both PmrAB and CpxRA directly regulate the transmissive-phase expression of Dot/Icm components and substrates which are essential for *Legionella* virulence [22,151,152]. The *L. pneumophila* CpxRA two component response regulators consist of the CpxR response regulator and the CpxA sensor histidine kinase [151]. The involvement of the CpxRA in *L. pneumophila* virulence is first identified as a direct regulator of the icmR gene [151]. Later, this two component response regulators are shown to participate in the regulation of two additional icm/dot genes (icmV and icmW), the lvgA gene and other 11 effector-encoding genes, and found to activate the expression of all the icm/dot genes it regulates, as well as five effector-encoding genes, and to repress the expression of six other effector-encoding genes [22].

The PmrAB two component response regulators consist of the PmrA response regulator and the PmrB sensor histidine kinase [153]. Previous study has shown a role for the PmrAB in regulating the expression of several genes encoding Dot/Icm-secreted effectors in *L. pneumophila* [153]. Later, the *L. pneumophila* PmrAB is found to activate the expression of many *L. pneumophila* effector-encoding genes including eukaryotic-like proteins, Dot/Icm apparatus and secreted effectors, type II-secreted proteins, regulators of the postexponential phase, stress response genes, flagellar biosynthesis genes that play special role in virulence [152]. The gene encoding for PmrA is shown to be required for intracellular growth of *L. pneumophila* in amoeba [153]. The environmental stimuli that activate the *L. pneumophila* PmrB sensor kinase is still unknown, but pH levels of the LCV might be related to the PmrB activation. To date, the *L. pneumophila* PmrAB regulon consists of 43 effector-encoding genes including some virulence genes, makes it the largest effectors regulon, and it includes approximately 15% of the known *L. pneumophila* effectors. Two groups of PmrAB regulated effectors with related functions arise. The first are three effectors (SidI, SidL, and Lgt3). They are found to interact with components of the eukaryotic translation elongation machinery (eEF1A and eEF1B). The interactions may lead to inhibition of host protein synthesis [154,155], thus contribute to bacteria virulence. The second group includes another three effectors (SdhA, SidF, and LegAU13/AnkB) and they seem to be involved in maintenance of the LCV in the host cell. Two of these effectors including SdhA and

SidF are shown to have anti-apoptotic activities [156,157]. Beside these two effectors, the LegAU13/AnkB which harbors an ankyrin domain and an F-box motif, is shown to produce polyubiquitinated proteins on the LCV, and degradation of these proteins supply amino acids required for bacterial growth [158]. In addition, LepB which is a known PmrAB regulated effector, is shown to function as a GAP for Rab1 and found to translocate into host cells and to perform its function several hours post-infection [102]. All of these informations about the PmrAB regulators and their related effectors highlighted the important role of these regulators in *Legionella* virulence.

The most important regulatory switch that controlling the replicative- to transmissive-phase cycle as well as in flagellar gene expression of *Legionella* is a two-component system, named LetAS [159-161]. The regulatory mechanism of LetAS is it acts through the derepression of CsrA [162]. Small noncoding RNAs that mediate this process have been identified recently [163,164]. The mechanism that noncoding RNAs involve in the mediation of this process can be illustrated as follow: the LetAS relays the signal which increases the expression levels of the small RNAs RsmY and RsmZ, these two noncoding RNAs possess multiple CsrA binding motifs that act to sequester the transcriptional repressor CsrA, and CsrA is released from its target mRNAs inducing the expression of transmissive traits and allow the transcription of genes that mediate the transmissive phenotype [163,164]. Many virulence gene expressions including the flagellar gene expression is thought to be regulated by this CsrA-dependent pathway [149].

Additionally, the LqsRS two component regulators include a response regulator LqsR which is shown to be RpoS depended, contributes to virulence processes such as phagocytosis and the formation of the LCV by relaying signals from the adjacent sensor kinase LqsS [144,165]. Similar to this system, an autoinducer synthase, LqsA, which is shown to synthesize 3-hydroxypentadecan-4-one, the small *Legionella* autoinducer 1 (LAI-1), is identified to be presumably recognized by the sensor kinase LqsS, which in turn probably activates LqsR [166]. Tiaden, A et al. showed that the LqsA mutant of *L. pneumophila* showed only a modest decrease in phagocytosis, while the overexpression of LqsA in an LqsR or LqsS mutant strain of *Legionella* could background restore the severe uptake defects of these mutants and their experiments also revealed that LqsR affects the expression of genes involved in virulence including 12 effector-encoding genes [144]. These results indicated that LqsA does have a role in modulating virulence and possibly through regulating the expression of genomic island LqsR or LqsS [167]. Recently, an 'orphan' homologue of LqsS termed LqsT was identified which probably also respond to LAI-1 [168]. In a transcriptome analysis of the Δ lqsA, Δ lqsS, and Δ lqsT mutants indicated that the expression levels of several other effector-encoding genes was changed in these mutants [167,168], further revealing the evidence that these regulator genes do have specific role in *Legionella* virulence. The cross-link regulation can be found between LqsRS and LetAS systems. The expression of LqsR was found to require the RpoS, and it was also found to be dependent to a smaller extent on the response regulator LetA [144]. Furthermore, the expression of LqsR was found to be regulated at a post-transcriptional level by the small RNAs RsmY and RsmZ and by CsrA which was regulated

by LetAS. These results indicate that these two two component regulators (LqsRS and LetAS) both involve in the regulation of effector gene expression and this cross-link regulation may play important role in virulence. We conclude the model of the two component response regulators that control the expression of the factors that related to *L. pneumophila* virulence in **Figure 2**.

Conclusion

A great deal of Legionella species and legionellosis cases have been studied over the past 39 years. More and more Legionella species have been identified to be associated with disease. But the ultimately important species is *L. pneumophila*. More and more researchers are engaged in discovering the novel mechanism by which this bacterium infects eukaryotic cells and how the virulence generates.

Although Legionella is an environmental organism, its innate ability to replicate inside eukaryotic cells and its capacity to inadvertently avoid regular pathogen control mechanisms in the host cells lead it to be an important accidental cause of community- and hospital-acquired pneumonia. The rapid advances made the understanding of Legionella virulence

factors or genes have resulted mostly from the availability of genome sequences that have uncovered unusual features of the pathogen, such as the presence of virulence determinants which have similar characters to eukaryotic proteins. The uncover of novel virulence factors or effectors and the depth understanding of known virulence factors of Legionella species will help us to understand the virulence evolution and emergence of the *L. pneumophila* as a major respiratory pathogen in 58 Legionella species. The ongoing and effective studies of Legionella virulence proteins, associated factors or genes and their effects on host cell biology, along with the regulator of these factors will continue to deepen our understanding and viewpoints of the roles that various host cell signaling and trafficking pathways play in resistance to the bacterial infection. All of these informations will help us better generating new insights on the pathogenesis of *L. pneumophila* when infecting human macrophages and the novel strategies for legionellosis control and therapy.

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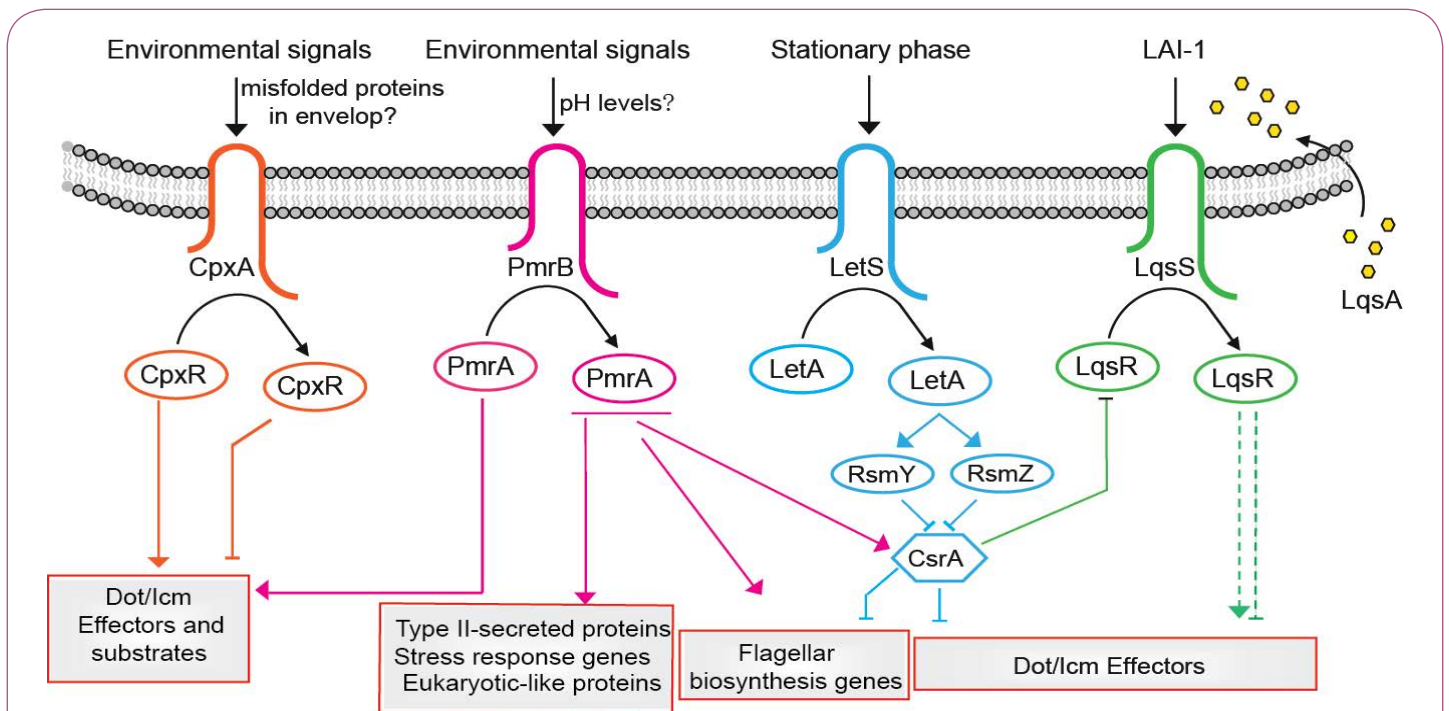


Figure 2 Models of four two-component response regulators that control the expression of the factors that related to *L. pneumophila* virulence. The environmental signals sensed by CpxA and PmrB may be mix folded proteins in envelop or pH levels, and the phosphorylation of these components is activated by transfer of the phosphate group to their cognate response regulators CpxR and PmrA respectively, which then directly activate or repress the transcription of their target genes. In stationary phase, LetAS activates the expression of the small RNAs including RsmY and RsmZ that sequester CsrA from its target mRNAs and relieve the CsrA post-transcriptional repression. The expression of *csrA* gene was also shown to be under the regulation of the PmrA, and LqsR was shown to be repressed by CsrA. The LqsS is activated after its binding to LAI-1 and, LqsR was shown to be repressed by CsrA. The way that LqsR affects Dot/Icm effector gene expression is still unknown. In this figure, solid lines and dashed lines indicate direct and indirect regulation, respectively; solid arrows and T-shaped symbols indicate activation and repression, respectively. The frames with red margin indicate virulence-related proteins, effectors or genes.

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