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Legionella and legionnaires´ disease: An overview

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ABSTRACT

Legionellosis is the generic term used to describe infections caused by different varieties of *Legionella* spp., including Legionnaires' disease (LD), a severe and potentially fatal form of pneumonia, and Pontiac fever, a self-limited flu-like illness. Legionellosis is usually acquired through inhalation or aspiration of aerosols containing *Legionella* spp. These bacteria can cause acute consolidating pneumonia in susceptible patients who are at an advanced age, have underlying debilitating diseases, or are immunodeficient. The main natural reservoir for *Legionella* is water and this pathogen colonizes many different natural and man-made freshwater environments such as water networks, cooling towers, and water systems in buildings and hospitals. In recent years, various laboratory diagnostic tests for *Legionella* infections have changed significantly. Although the sequencing method is nowadays considered the fastest and most reliable method for differentiation and detection of different *Legionella* species, the isolation of these bacteria from clinical specimens is the golden standard for diagnosis of Legionnaires' disease. Today the urinary antigen test as the most rapid and inexpensive method is routinely used for diagnosis of LD caused by *Legionella* pneumophila serogroup 1. The macrolides and fluoroquinolones are still the mainstays for the treatment of *Legionella* infections. For the prevention of spreading the contaminated water aerosols and controlling *Legionella* infections, an effective water treatment procedure is necessary. This review describs and summarizes the latest available information about all aspects of *Legionella* and Legionnaires' disease.

1. Introduction

In June 1976, an unprecedented outbreak of pneumonia occurred among approximately 4400 American retired soldiers who attended the 58th Annual Convention American Legion at a downtown Philadelphia hotel on Broad Street. Two hundred and twenty-one people encountered the clinical criteria for respiratory syndrome, and 34 of them died[1]. The epidemiological studies conducted by US Centers for Disease Control (CDC), as well as health workers in Pennsylvania were not able to quickly diagnose the cause of the outbreak. The final hypothesis was that the cause of the outbreak was the air of the Bellevue-Stratford Hotel cooling units and air conditioning because the victims were among the people who had stayed at the hotel, but this theory has never been fully proved.

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After six months, in December 1976, Dr. Joseph McDade of CDC researchers, using the technique of guinea pig inoculation, was able to isolate the bacterium which caused the outbreak and identified it as a fastidious Gram-negative rod that was afterward named *Legionella* (L.) *pneumophila* (*L. pneumophila*). This name indicated both its victims and the newly described Legionnaires' disease (LD). Afterward, more outbreaks of LD occurred in the U.S. and other countries after 1976, which were emanated from contaminated water systems[1-3].

2. Taxonomy

The family Legionellaceae consists of the single genus *Legionella*. At this time, the family of Legionellaceae comprises over 60 species with 70 serogroups. Approximately 30 species were isolated at least once from patients and have thus been documented as pathogenic for humans. The number of identified species and serogroups of the genus *Legionella* remains to increase[4,5]. Most of the LD cases (nearly 95%), is caused by *L. pneumophila*, particularly *L. pneumophila* serogroup 1 that is responsible for 84% of the cases worldwide followed by *L. longbeachae* (3.9%) and *L. bozemanii* (2.4%), and other involved species, with less frequently, are *L. micdadei*, *L. dumoffii, L. feeleii*, *L. wadsworthii* and *L. anisa* (2.2% in total). There are 16 serogroups of *L. pneumophila*, and two each in *L. bozemanii*, *L. longbeachae*, *L. londiniensis*, *L. feeleii, L. hackeliae*, *L. sainthelensi*, *L. spiritensis*, *L. erythra*, and *L. quinlivanii*, and a single serogroup in each of remaining species (Table 1)[6,7].

3. Microbiology

In general, the *Legionella* spp. are small Gram-negative bacilli that range from 0.3 to 0.9 μm in width and from 2 to 20 μm in length[8,9]. They are non-spore forming, unencapsulated, catalase-positive, urease-negative, and usually, have limited motility. The bacterium has one or more polar, subpolar, and lateral flagella. In contrast to most Gram-negative bacteria, *Legionella* spp. contain high amounts of branched-chain fatty acids in the cell wall that make cell staining difficult. *Legionella* is strictly aerobic and has a respiration metabolism that utilizes amino acids for energy, and requires iron salts as well as *L*-cysteine-HCl for growth on laboratory media[9]. Their colonies are usually detectable after 3 to 5 d of incubation on various selective and non-selective media. When first visible, the young colonies are 0.5-1.0 mm in diameter, granular or speckled opalescence, self-contained, convex, smooth, with a ground-glass appearance and glistening hue[10].

Table 1. *Legionella* species and serogroups associated with human disease.

Legionella species	Sero-groups	Association with human clinical cases
L. adelaidensis	1	Unknown
L. anisa	1	(L, P)
L. beliardensis	1	Unknown
L. birminghamensis	1	L
L. bozemanii	2	L
L. brunensis	1	Unknown
L. busanensis	1	Unknown
L. cherrii	1	Unknown
L. cincinnatiensis	1	L
L. drancourtii	1	Unknown
L. drozanskii	1	Unknown
L. dumoffii	1	L
L. erythra	2	Unknown
L. fairfieldensis	1	Unknown
L. fallonii	1	Unknown
L. feeleii	2	L, P
L. geestiana	1	Unknown
L. gormanii	1	L
L. gratiana	1	Unknown
L. gresilensis	1	Unknown
L. hackeliae	2	L
L. israelensis	1	L
L. jamestowniensis	1	Unknown
L.jeonii	1	Unknown
L. jordanis	1	L
L. lansingensis	1	L
L. londiniensis	2	Unknown
L. longbeachae	2	L
L. lytica	1	L
L. maceachernii	1	L
L. micdadei	1	L, P
L. moravica	1	Unknown
L. nautarum	1	Unknown
L. oakridgensis	1	L
L. parisiensis	1	L
L. pneumophila	16	P,L
L. quateirensis	1	Unknown
L. quinlivanii	2	Unknown
L. rowbothamii	1	Unknown
L. rubrilucens	1	Unknown
L. sainthelensi	2	L
L. santicrucis	1	Unknown
L. shakespearei	1	Unknown
L. spiritensis	2	Unknown
L. steigerwaltii	1	Unknown
L. taurinensis	1	Unknown
L. tucsonensis	1	L
L. wadsworthii	1	L
L. waltersii	1	Unknown
L. worsleiensis	1	Unknown

L.: *Legionella*; L: Caused pneumonia; P: Caused Pontiac fever.

4. Ecology

The genus of *Legionella* is ubiquitous in natural and artificial aqueous environments worldwide and can survive in varied environmental conditions. Water is the major reservoir for *Legionella* and the bacteria are found in freshwater environments worldwide.

The only exception in this regard is *L. longbeachae*, that is often isolated from potting soil[11]. The *Legionella* spp. have been isolated from hot water systems up to 66 ℃. However, the optimum growth temperature is 35 ℃ (ranging from 20 ℃ to 42 ℃)[12]. Tolerance to chlorination in *Legionella* spp. have increased, and thus it can multiply in thermal water sources, including cooling towers, water softener, showerheads, whirlpool spas/hot tubes, holding tank or cistern, and respiratory ventilators[13].

The organisms colonize and persist in biofilms on the surfaces of these systems, wherein they are lesser sensitive to the effects of chlorine and various biocides[14]. *Legionella* has evolved to persist and multiply in various environmental niches, such as biofilms, nematodes, and within free-living protozoa[15]. When *L. pneumophila* grows inside protozoa, HeLa cells, epithelial cells or even clinical specimens, it differentiates to a highly infectious, metabolically-resting, and morphologically distinct cyst-like form which termed as mature intracellular form that is exclusive to the intracellular milieu and cannot grow *in vitro*[16].

5. *Legionella* in biofilm

There are restricted data about chemical and biophysical conditions, as well as the molecular mechanisms that allow the growth of *Legionella* in biofilms[16]. Most recent evidence suggested that the growth of *Legionella* in biofilms may has a role in increasing the pathogenicity. Serogroups 1, 10, and 12 of *L. pneumophila* that were isolated from biofilms had more cytotoxicity for the amoeba than reference and epidemic outbreak strains[17]. Furthermore, preliminary findings suggest that biofilm-derived *L. pneumophila* strains can escape from the innate immune response in macrophages[16]. Some bacterial species stimulate the persistence of *L. penumophila* in biofilm while others exhibit inhibitory effects. For example, *Klebsiella pneumoniae*, *Flavobacterium* sp., *Empedobacter breve*, *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Pseudomonas aeruginosa* can stimulate the persistence and presence of *L. pneumophila* in biofilms[18].

6. Pathogenesis

Legionella species are considered opportunistic pathogens, which accidentally cause disease in humans. The life cycles of *Legionella* have been characterized in both protozoa and mammalian cells. The pathogenic mechanisms involved in survive and replication in their protozoal host, are resembled those observed in human cells, especially in respiratory epithelial cells and alveolar macrophages[19]. The infection in humans is initiated by direct inhalation or microaspiration of fine aerosol containing both virulent and non-virulent strains. Once within the alveoli, the virulent strains are taken up by alveolar phagocytes where they multiply and inhibit the fusion of phagosomes with lysosomes and acidification of the phagosome[20]. The bacterial growth within infected macrophages has been estimated at 100 to 1000 fold within 48 to 72 h of infection, which is considered remarkable compared to other intracellular opportunistic bacteria (*e.g.*, *Salmonella*, *Mycobacterium*, *Listeria*)[21].

After adequate intracellular multiplications, the bacteria kill alveolar macrophages by either apoptosis or necrosis mediated by a pore-forming activity or both, and then transfer into the extracellular environment, which can infect other macrophages[22]. As a result of this intracellular multiplication, macrophages, neutrophils, and peripheral blood monocytes penetrate the alveoli and capillary leakage and leads to severe inflammatory response and edema. Not all *Legionella* species are able to infect macrophages. However, *L. pneumophila* that possesses significant virulence factors can infect macrophages and replicate within various protozoa found in soil and in water[23]. During the process of *Legionella* phagocytosis, a complex cascade of processes occur, such as interdiction of phagosome-lysosome fusion, decrease of phagosome acidification, prevention of the oxidative burst, alteration in organelle trafficking and inhibition of phagosome maturation[24]. In contrast to common symmetrical and conventional uptake of pathogens, *L. pneumophila* Philadelphia-1 strain is phagocytosed by macrophage through an exclusive uptake process called "coiling phagocytosis". This process initiates through binding of C3b component of complement to a purin protein in the bacterium outermembrane, and then subsequent binding to complement CR1 or CR3 receptor on surface of phagocytes will occur. In this condition, the pathogen is swallowed through asymmetrical engulfment by a unilateral pseudopod that coils around the bacterium[25]. Some investigations have ascertained that the other serotypes of *L. pneumophila* and non-*L. pneumophila* species can enter host cells by conventional phagocytosis[26].

L. longbeachae is another causative agent of LD which together with *L. bozemanae*, and *L. micdadei* accounts for approximately 2%-7% of *Legionella* infections worldwide[24]. Unlike *L. pneumophila*, *L. longbeachae* is mainly found in soil pots and transmitted by inhalation of contaminated soil dust[17]. *L. longbeachae* virulent strains exhibit the same intracellular phenotype as *L. pneumophila*, with the replicative vacuole associated with rough endoplasmic reticulum and dotted with ribosomes[24].

7. Virulence factors

The main feature of the *Legionella* pathogenesis is its ability to proliferate intracellularly. But the whole infection process in both protozoa and mammalian cells is included bacterial cell attachment to host cells, survival, intracellular replication, and cell-to-cell spread which all specify its pathogenesis[27]. Various specific virulence factors encoded by several pathogenicity islands and specific antigens have been described for genus *Legionella*[28-32]. The direct evidence in some studies showed that the distribution of virulence genes in different types of *Legionella* strains such as reference and environmental *L. pneumophila* strains were much higher than

those in reference and environmental non-*L. pneumophila* strains. respectively. Furthermore, clinical *L. pneumophila* strains maintained more virulence genes compared to other types of *Legionella* strains. Diverse distribution patterns between reference and environmental non-*L. pneumophila* strains may also disclose 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[32].

Macrophage infectivity potentiator protein, a 24-kDa surface-exposed protein, is demonstrated as a virulence factor in *L. pneumophila* that is necessary for optimal intracellular survival. This protein is specific expressed on the surface of *Legionella* and displays peptidylprolyl *cis/trans* isomerase activity that is required for invasion of macrophages, and transmigration through an *in–vitro* lung-epithelial barrier. Furthermore, macrophage infectivity potentiator is a moonlighting protein that binds to collagen type Ⅳ and thus enables efficient colonization and dispersal of the *L. pneumophila* to transmigrate across the epithelial cell barrier[33].

The *Legionella* type Ⅳ secretion system called intracellular multiplication/defective for organelle trafficking is essential for intracellular replication, conjugating and injecting plasmid DNA or toxins into host cell cytosol. This system inhibits host cell apoptosis and also regulates the host vesicular transport to evade delivery to lysosomes and thus enhances vacuole alteration through the host vesicles in the primitive secretory pathway[34]. The *dot/icm* genes comprise 25 genes in two distinct regions of the *Legionella* chromosome. Region Ⅰ contains seven genes (*dotDCB* and *dotA–icmVWX*) and region Ⅱ includes 18 genes (*icmT*, *S*, *R*, *Q*, *P*, *O*, *N*, *M*, *L*, *K*, *E*, *G*, *C*, *D*, *J*, *B*, *F*, *H*). *Legionella* operates this system to convey essential virulence factors required for the initiation of the infectious process, such as intracellular multiplication, to modulate anti-apoptotic host cell signaling pathways, to degrade the phagosome membrane, and to disrupt host cell membranes for the bacterial release in extracellular environment. Any type of mutations in *dot/icm* locus leads to loss of virulence[34,35].

Iron depletion decreases the ability of *L. pneumophila* and other organisms for replication. *L. pneumophila* obtains iron during growth in the macrophage by a factor named more regions allowing vacuolar colocalization N (MavN), a protein translocated by *L. pneumophila*'s type Ⅳ secretion system. MavN is required for intracellular growth, but not for growth in media and is highly upregulated during iron starvation. Another virulence factor of *L. pneumophila* is the *rpsL*, a secreted effector, activates caspase-3 and host cell apoptosis. Other potential virulence factors include heat shock proteins, zinc metalloprotease, cytotoxins, lipopolysaccharide and phospholipases[34,36].

8. Epidemiology

The precise geographical variation of the incidence of LD in many countries throughout the world remains unrevealed, mostly due to the difference in monitoring systems, knowledge levels and diagnostic methods[37]. According to the European Centre for Disease Prevention and Control, the rate of legionellosis in 2014 among European countries reached 13.5 cases per million inhabitants, which represents a significant increase in the incidence of legionellosis. The rate of notification among European countries ranged from less than 0.1 per million people in Romania and Bulgaria to 56.4 per million in Portugal[38].

Travel is an unfamiliar factor in the attainment of legionellosis in the society and roughly 20% of reported cases of LD are associated with traveling. Incidence rates and estimated risk to travelers differ by country and only 4% of cases were related to a known outbreak[39].

LD accounts for 2%-15% of hospitalized cases of community-acquired pneumonia[40]. Most of the cases recorded in the U.S. are sporadic. In the 1990s, explanatory epidemiological information released by the CDC documented 8000 to 18000 hospitalized cases of community-acquired LD[41,42].

Regarding that many countries lack proper methods of diagnosing the infection or adequate surveillance systems, the incidence of the disease is unknown worldwide[43]. However, in some countries such as Japan, China, South Korea, Iran, South Africa, and Colombia, several studies have been conducted upon patients with respiratory infections as well as various sources of water indicating the extensive incidence of *Legionella* especially *L. pneumophila* serogroup 1[44-49].

Well-known risk factors for community-acquired and travel-associated legionellosis include long-term smoking, chronic lung disease, organ transplants, age >50 years, diabetes, chronic respiratory or heart disease, weakened immune system (glucocorticoid treatment), hematologic malignancies, lung cancer and heavy consumption of alcoholic beverages[50]. LD is mostly transmitted from the environment *via* inhalation of infectious aerosols or microaspiration of water comprising *Legionella* spp. The other less common means of transmission include direct inoculation into the lungs through respiratory therapy equipment, massive aspiration of contaminated water into the lungs through near-drowning and surgical wound contamination with tap water[51]. Until recently there was no evidence of animal to human or human to human transmission exists, however, new cases of person to person transmission have been reported[52,53].

Legionella has been found in multiple natural and artificial aquatic environments including fish ponds, cooling tower, water fountain, hot tubs, water spa, industrial equipment, hot and cold water systems, domestic plumbing systems, whirlpool spas, and also hospital equipment such as nebulizers, respiratory devices, and nasogastric tubes[54]. The mortality rate of legionellosis is highly variable (1%-80%), depending on the duration of diagnosis and specific treatment, the patient's underlying disease, and whether the disease is nosocomial, sporadic, or a portion of an outbreak[55].

9. Clinical features

The clinical manifestations of legionellosis comprise the severe multisystem disease with consolidating pneumonia (LD) or Pontiac fever and extrapulmonary in fections[56]. Furthermore, many people who are infected with *Legionella*, as confirmed by seropositive, will remain asymptomatic[57]. The average incubation time of LD is between 2 and 10 d[58]. This syndrome was often initially characterized by fever (high grade), weakness, anorexia, malaise, fatigue, and lethargy; also, patients may progress a mild and non-productive cough[59,60]. The sputum may be blood-streaked, and hemoptysis (cough up blood) may exist. Pleuritic chest pain is prominent in some individuals, and if associated with hemoptysis may lead to a mistaken diagnosis of pulmonary embolism (blood clots in the lungs)[61]. Abdominal pain and gastrointestinal symptoms such as abdominal pain, nausea, vomiting, and watery diarrhea are typically prominent in many patients[62]. Approximately half of patients suffering from neurologic disorders, such as headache, delirium, confusion, stupor, agitation, and hallucinations[63].

A number of non-specific laboratory findings that are common include hyponatremia, decreased serum phosphorus, hypophosphatemia, elevated liver-associated enzymes, increased creatine kinase (MM fraction), leukocytosis or leukopenia, thrombocytopenia, high erythrocyte sedimentation rate and C-reactive protein levels, disseminated intravascular coagulation, serum ferritin promotion, elevated lactate dehydrogenase, myoglobinuria, pyuria, and microscopic hematuria[64].

Besides *L. pneumophila*, other *Legionella* species have been known as human pathogens, according to their isolation from clinical samples. The clinical and radiographical findings of non-*L. pneumophila* species do not differ significantly, from those caused by *L. pneumophila*. In some rare cases, the *Legionella* spp. may be disseminated to other organs through the bloodstream and the lymphatic system. However, the most common non-respiratory manifestations include sinusitis, cellulitis, peritonitis, pyelonephritis, pancreatitis, splenomegaly and spleen rupture, pericarditis, myocarditis, wound infections, endocarditis, arthritis, and central nervous system infections[65,66]. Pontiac fever is a self-limited, non-fatal illness with a 5 h to 3 d (most commonly 24-48 h) incubation period and a short duration. This influenza-like illness usually diagnosed only during an outbreak of the disease and associated with exposure to *Legionella*. The term of Pontiac has been originated from a city with this name in Michigan, which was the place of an outbreak in 1968. Its exact pathogenesis remains unknown and there is no agreed-upon definition, however, the main symptoms include fever, chills, myalgia, headache, malaise, arthralgia, non-productive cough, abdominal pain, fatigue, and a sore throat. Pontiac fever has been recently documented lesser frequently than before, and even patients without antimicrobial therapy and without complications, would have complete recovery only within two to five days[67,68]. Another aspect of LD is its importance in transplant recipients. Immunocompromised patients such as organ or bone marrow transplant recipients have the highest risk of the nosocomial LD. It has been shown that heart and liver transplant patients have an elevated incidence of LD. *Legionella* spp. are among the most prevalent pathogens in certain diseases in recipients of liver transplants, probably associated with simultaneous splenectomy for related hypersplenism[67].

10. Laboratory diagnosis

The clinical symptoms and radiological features of the LD patients are nonspecific and can not be distinguished from other types of pneumonia[69]. Accurate laboratory confirmation is, therefore, essential for the identification of *Legionella* and appropriate treatment. At present, several laboratory tests are used for the recognition of *Legionella* in clinical samples including:

(1) isolation of the causative organism from clinical specimens such as respiratory secretions, blood, and biopsy of tissue;

(2) detection of the bacterium in respiratory secretions or lung tissue by immunofluorescent microscopy, *e.g.* direct fluorescent antibody test (Table 2)[70];

(3) detection of *Legionella* antigens in urine specimens[69,71];

(4) serological and antibody-based assays *e.g.* indirect immunofluorescent assay and enzyme-linked immunosorbent assay[72];

(5) detection of *Legionella* spp. nucleic acid in respiratory secretions using molecular methods *e.g.* polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP)[73];

(6) matrix-assisted laser desorption/ionization-time of flight mass spectrometry[74].

Culture and isolation of *Legionella* spp. from expectorated sputum and respiratory secretions, which has a specificity of 100%, is still the gold standard for LD diagnosis[75]. Although culture requires special media, adequate processing of samples, and technical expertise, the routine use of this method is highly recommended because it enables the identification of all *Legionella* species, research on the outbreak, or even antibacterial sensitivity testing[66]. The standard media used to culture *Legionella* spp. from clinical specimens is buffered charcoal yeast extract agar supplemented with -ketoglutaric acid, dyes and antimicrobial agents (*i.e.* vancomycin, anisomycin, and polymyxin B). Despite this medium is available commercially and can be easily prepared, many clinical microbiology laboratories have neither the proficiency nor the capability to properly perform these specialized cultures[76].

Direct immunofluorescence assays are often used for rapid detection of *Legionella* spp. in respiratory and tissue specimens, although the sensitivity of this test is low, technically demanding, and false-positive results may occur due to cross-reactions with other bacteria[77].

The urinary antigen testing has considerably outpaced other

Table 2. Main methods for laboratory diagnosis of *Legionella* infection.

Diagnostic method	Sample	Specimen collection time	Information offered by positive results	Sensitivity (%)	Specificity (%)	Notes
Culture and isolation	Sputum, lower respiratory tract secretions, lung biopsy, pleural fluid, bronchoalveolar lavage, blood, extrapulmonary tissues, synovial/joint fluid	3-14 d	Detection of bacteria related to the *Legionella* genus	<10-80	100	The gold standard and diagnostic test of choice for detection of all species and serogroups
Detection of urinary antigen	Urine	3-4 h	Only for *L. pneumophila* serogroup 1 infection	70-90	>95	Easy, very rapid; frequently earliest positive result; may remain positive for several days to months
Serological testing	Paired serum	3-10 weeks	Detection of increase in antibody titers against some *L. pneumophila* serogroups	40-80	>95	Acute- and convalescent-phase serum samples collected 3-10 weeks separately; interpretation of a single titer result can be misinforming
Direct fluorescent antibody test	Same as culture	2-4 h	Discrimination between *Legionella* serogroup and/or species	25-75	>95	Very rapid; experience needed; sensitivity constantly less than for culture; maximum specificity with monoclonal antibody
Molecular amplification (PCR)	Sputum, lower respiratory tract specimen, serum, urine	4-6 h	Identification and typing of all *Legionella* species and serogroups	30-100	>95	Rapid; not well standardized; sensitivity and specificity dependent on the design of the methodology and sample source
MALDI-TOF mass spectrometry	Culture isolate	~15 min	Identifying bacteria as *Legionella* spp.	90-99	Unknown	Rapid; inexpensive; requires high-tech and expensive equipment; requires a pure-culture isolate; sensitivity depending on species examined

Adapted from reference 76.

laboratory methods for diagnosis of LD, and currently, 97% of clinical diagnoses are acquired by utilizing this test due to its speed, simple procedure, easiness of sample collection, low cost and commercial accessibility[78,79]. The test is positive within 24-72 h of clinical symptom onset and may persist positive for some weeks or months despite inadequate antibiotic therapy. Two popular formats of the test include the enzyme immunoassay and immunochromogenic test[80]. The limitations of the urinary antigen testing consist of poor sensitivity for other non-*L. pneumophila* species, in spite of its high specificity for *L. pneumophila* serogroup 1[81]. In addition to urinary antigen detection, molecular methods such as a real-time PCR testing that targets the 23S-5S rRNA intergenic spacer region can be used for rapid detection, and this assay has the ability to differentiate *L. pneumophila* from other non-*L. pneumophila* species[79].

Of the several antibody detection methods that have been established to detect antibodies to *Legionella* spp., enzyme-linked immunosorbent assay and indirect immunofluorescent assay are the most commonly used[82]. Although the serological methods have advantages such as the ability to perform in case of impossibility of culturing, cheapness, valuable for retrospective epidemiological studies and usefulness for epidemiological studies in outbreaks, they have generally been superseded by other tests because of the length of time required, delayed seroconversion, and their need for paired sera. Furthermore, obtaining appropriate convalescent samples is difficult for these methods, and cross-reactive antibodies may be occasionally observed in patients with infections caused by other respiratory pathogens[83]. CDC laboratories also do not carry out serological tests to detect legionellosis, due to limitations of these approaches[84].

Molecular techniques can expand diagnosis since they allow detecting all *Legionella* species and because of their greater sensitivity than other methods. DNA amplification by PCR, especially real-time PCR has been used to detect *Legionella* DNA, from both environmental and clinical specimens including sputum, bronchoalveolar lavage, blood, and urine[85]. PCR can amplify very small amounts of *Legionella* DNA, providing results within the shortest time and has the potential to identify infections caused by any *Legionella* species[86]. The sensitivity and specificity of *Legionella* PCR dependent on the design of the methodology and sample source, for example, when testing samples from the lower respiratory tract, the sensitivity is 80% to 100% and specificity is close to 100%[87]. PCR, specifically real-time PCR, has been characterized to the detection of the *L. pneumophila* DNA using several different genes including ribosomal RNA (*16S rRNA*, *23S rRNA*, *5S rRNA*), RNA polymerase (*rpoB*), heat-shock protein (*dnaJ*), and macrophage infectivity potentiator (*mip*)[88,89]. Although the PCR method is a rapid assay to confirm *Legionella* infections, and also several commercial kits are available, however only one of them is approved by Food and Drug Administration, yet not marketed[90]. LAMP, or loop-mediated isothermal amplification, is a process similar to PCR for the detection of all *Legionella* spp., but requires shorter time for processing. The LAMP technique is highly specific for differentiation of *L. pneumophila* from other *Legionella* spp. without the need for complicated equipment and post-amplification procedures[91]. In the last years, specific genes sequencing evaluation has been used for the taxonomic analysis of *Legionella* spp. *Legionella* species identification has relied mainly on *16S rRNA* gene or *mip* gene sequencing, which has considerably improved the information about the evolution, pathogenicity and genetic diversity of this bacteria[11,79].

In recent years, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has emerged as a simple, rapid and relatively inexpensive method for identification of numerous microbes, including various *Legionella* species and strains[92,93]. In the MALDI-TOF MS method the bacterial species are identified based on their specific protein patterns[94]. Disadvantages of this technique include inappropriate as a first-line rapid identification test for primary samples because culture isolation is required for diagnosis, the impossibility of serogrouping of strains, and FDA-approval is not available yet[95].

The diagnosis of Pontiac fever is usually based on its epidemiological, clinical laboratory, and environmental microbiological findings. If epidemiologic and clinical investigations provide an acceptable indication of *L. pneumophila* serogroup 1 associated Pontiac fever, the urinary antigen testing can then be used to confirm the cases. The *Legionella* urinary antigen testing is the most broadly used diagnostic test for the diagnosis of LD, but based on previous studies, this test for Pontiac fever has not been useful, as none of the patients in four different Pontiac fever outbreaks associated with *Legionella* species had positive test for *L. pneumophila* serogroup 1 antigenuria. Detection of Pontiac fever may be a sign of contaminated with environmental *Legionella* and the risk of serious pneumonia[96-98].

11. Treatment

Since LD is considered by many complications and co-morbidities, delay in starting suitable treatment could be associated with increased mortality. At present, the mortality rate of LD has decreased, due to an increase in the suspicion index by physicians, initial empirical treatment with antibiotics that cover all *Legionella* strains and the emergence of rapid laboratory tests[99].

Given the fact that LD does not have any distinguishing clinical manifestations, an effective empirical therapy against *Legionella* spp. is required in the initial controlling of all community and hospital-acquired pneumonia infections until one of the specific microbiological diagnoses prepared[100,101].

The reports showed that antimicrobial agents that achieve therapeutic intracellular concentrations within alveolar macrophages were more clinically effective than antibiotics with poor intracellular penetration. Therefore, the commonly used antibiotics with demonstrated clinical effectiveness in LD were macrolides,

tetracyclines, quinolones, doxycycline, rifampin, trimethoprim-sulfamethoxazole, and tigecycline. These antibiotics that had relatively high intracellular penetration, have been used alone or in combination[102-104]. Today, erythromycin the first well-established macrolide for treatment of LD is less prescribed compared to newer ones because of its side effects (especially phlebitis and gastrointestinal intolerance)[105].

In recent years, the newer macrolides (azithromycin, clarithromycin) and other groups of drugs such as fluoroquinolones (levofloxacin, moxifloxacin, gatifloxacin) and a new glycylcycline drug, have been prescribed for the treatment of LD in healthy and immunocompromised individuals, and are licensed by the FDA[106,107]. Referring to the dose and route of administration of antimicrobial agents, whether intravenous or oral, should be determined according to the severity of the disease, underlying risk factors, and the availability and potential toxicity of individual drugs[108,109].

The Infectious Diseases Society of America/American Thoracic Society Guidelines recommended that for patients with a suitable clinical response, 7-10 d antibiotic therapy can be sufficient. The duration of treatment should be determined individually and calibrated with clinical response and improvement in biomarkers such as CRP and procalcitonin. However, therapy duration may need to be extended to 21 d for severe situations and/or immunocompromised patients. Complications such as lung abscesses, empyema, and extrapulmonary infection like endocarditis or meningitis, might need longer therapy[110-112].

Because of the absence of pneumonia, short duration of illness, and the mild nature of symptoms, antimicrobial treatment for Pontiac fever is usually not needed. Like other pneumonic diseases, supportive treatment is also performed in the form of oxygen, intravenous fluids, and chest physiotherapy for Pontiac fever[113].

12. Prevention

Control and prevention of legionellosis re quires identification of the potential sources of organisms and reducing the production of water aerosols in the environment that may be contaminated with *Legionella* spp. [14].

There are at least two most appropriate and cost-effective strategies to prevent nosocomial legionellosis. The first approach is the periodic routine culturing of drinking water samples of the hospitals to detect *Legionella* species. The second method for controlling nosocomial legionellosis includes maintenance of an index of the suspicion for LD, proper use of diagnostic tests for legionellosis in patients with nosocomial pneumonia and those who are at risk of developing the disease and die from infection, initiating of research for a hospital source of *Legionella* species upon identification of nosocomial infection, routine maintenance of cooling towers and utilizing sterile water for the filling and terminal washing of nebulizer devices[114].

A number of *Legionella* growth control methods in drinking water supply systems include thermal methods (superheat and flushing), ultraviolet light sterilization, hyperchlorination, ozonation, copper-silver ionization, and instant steam heating systems[115,116]. Since the bacteria in the biofilm are more resistant to biocides and heat treatment than freely circulating bacteria, therefore, reducing *Legionella* in the biofilms is an important control measure against the proliferation of this bacteria and may lead to the most efficacious control proceedings which can help in preventing legionellosis[117-119].

Thus far, effective vaccines that can prevent legionellosis are not available[120]. Pontiac fever is usually described in the epidemic settings, indicating environmental contamination by *Legionella* species. Therefore, its identification may be a rapid preventive measure to stop the outbreak of LD[79].

13. Conclusions

LD is an often severe and potentially fatal form of bacterial pneumonia if treatment is not taken in time. Although most cases of LD occur sporadically, outbreaks can occur both in the community and in hospital. LD is mostly transmitted from the environment through inhalation of contaminated aerosols or microaspiration of contaminated water. Legionellosis is characterized by various manifestations and clinical symptoms such as fever and other organ-specific symptoms and signs. The most important diagnostic tests for detection of *Legionella* infection in the early stages of the disease include sputum culture, PCR testing of respiratory secretions, and urinary antigen tests. Now testing of urine for *Legionella* antigen in combination with standardized PCR assays will be major advances in *Legionella* diagnostics. Fluoroquinolones and newer macrolides are appropriate for the treatment of LD in healthy and immunocompromised individuals. The most appropriate and cost-effective way to control and prevention of LD is the maintenance of water systems that may be contaminated with *Legionella*, as well as eradication of biofilms.

Conflict of interest statement

The authors report no conflict of interest.

Authors' contributions

All authors contributed to collect of data, prepare of primary draft or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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