

Nosocomial Legionnaires' Disease: Risque and Prevention

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Abstract In 1977, Fraser et al. described an outbreak of pneumonia among legionnaires attending a convention at a hotel in Philadelphia in 1976. Legionnaires' disease (LD) can be nosocomial, community acquired or travel related. The incidence of hospital-acquired legionellosis appears to be increasing. Colonization of water systems by *Legionella* spp. is ubiquitous in hospitals throughout the world. The outbreak, which later became known as legionnaires' disease, was caused by a new pleomorphic, faintly staining gram-negative bacillus, *L. pneumophila*, which was isolated at the Center for Disease Control from lung tissues of legionnaires who died. Risk assessment for this disease forms the basis for the institution of control measures. Detection and quantification of *Legionella* spp. in the environment, in particular in the hospital water distribution system is one of the cornerstones of risk assessment. This review summarizes the current state-of-the-art regarding these aspects and points out important areas which require further study. The environmental surveillance revealed that the centralized hot water distribution system of the hospital was colonized with *Legionella*. Methods of prevention of the organisms for eradication involved in hospital water systems.

Keywords Legionnaires' Disease, Nosocomial, *Legionella Pneumophila*, Environment, Risk Assessment, Prevention

1. Introduction

Hospital-acquired pneumonia (HAP) is considered the second most frequent cause of nosocomial infection, accounting for 15 to 20% of these infections[1]. Nosocomial Legionnaires' disease has become increasingly common, contributing up to 30 percent of hospital-acquired pneumonias in some institutions[2]. Legionnaires' disease, the pneumonic form of legionellosis, usually is acquired by inhalation or aspiration of legionellae from contaminated environmental sources. Potable water is an important source of both nosocomial and community acquired *Legionella* infections[3]. Nosocomial Legionnaires' disease is an important problem in some hospitals in the world. It has been estimated that 20–30% of legionellosis are nosocomial infections and that they are associated with a contamination of the hospital's water distribution system[4]. Colonization of hospital water distribution systems with *L. pneumophila* serogroup 1 has been repeatedly linked to acquisition of nosocomial Legionnaires' disease[5].

Obviously, the hospital/facility-related outbreaks do not have a seasonal distribution as with community acquired Legionnaire's disease[6]. *Legionella* is an opportunistic pathogen with widespread distribution in the environment.

Numerous reports have demonstrated that the major sources for Legionnaires' disease are the potable water systems of large buildings including hospitals, nursing homes, and hotels. *Legionella* is a common cause of hospital acquired pneumonia, especially for immunocompromised patients[7]. An epidemiological investigation should be initiated in the event of microbiologically confirmed *Legionella* pneumonia in a patient, who was admitted to the hospital 2-10 days before the onset of illness, or if two or more patients are infected attending an outpatient clinic. Legionellosis of human intervention of the environment is the cause of emerged, since *Legionella* species are found in aquatic environments, and thrive in warm water and warm, damp places, such as cooling towers.

2. History of Hospital-acquired legionnaires' Disease

The earliest recognized community outbreak, diagnosed retrospectively, was in Austin, Minnesota in 1957[8] and the first reported outbreak of hospital acquired Legionnaires' disease was in a psychiatric hospital at St Elizabeth's in Washington DC in 1965[9]. The hospital housed 6000 patients in multiple buildings on a 350 acre (1.4 km²) campus, in which 81 patients contracted pneumonia, with 15 deaths. However, analysis of stored serum specimens in 1977 showed that 19 of 26 patients tested seroconverted to *L. pneumophila* serogroup 1. In July 1968, 144 visitors or personnel of a county health department in Pontiac, Michig

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an contracted an acute febrile myalgia[10]. The responsible agent was retrospectively determined to be *Legionella*, and the name given to this self-limited version of disease was "Pontiac Fever". Subsequent Legionnaires' disease epidemics have rarely implicated soil excavation, although disruptions of potable water systems and contamination of building plumbing systems during construction have been alternative explanations[11]. An interesting historical note is that the attempted assassin of US President Ronald Reagan is currently confined to this institution[12]. The largest outbreak of hospital acquired Legionnaires' disease occurred at the Wadsworth Veterans' Administration Medical Center (VAMC) in Los Angeles, with at least 218 confirmed cases from 1977 to 1982[13]. Since then, more than 300 reports of hospital-acquired Legionnaires' disease have appeared in peer-reviewed literature and public-health reports[13]. An outbreak of a pneumonia-type human disease in 1976 was found to have originated from a hotel cooling tower at the American Legion Convention in Philadelphia. Thus, the name was established as the Legionnaires' disease[14]. The cause of this outbreak was identified by the Centers for Disease Control (CDC), Atlanta, as bacteria. *L. pneumophila* has been recognized as an important cause of community and nosocomial pneumonia. Legionnaires' disease is known to cause hospital acquired pneumonia and may occur as part of an outbreak or sporadically[15, 16]. Outbreaks are worldwide and have been reported from India[17], Turkey[18], Italy[19] Taiwan[20, 21] and Poland[22]. These outbreaks are usually due to aspiration of contaminated drinking water, but an oxygen humidifier[23] and a decorative fountain were implicated in two reports. Eight cases occurred in a hospital that had installed a decorative water fountain in the lobby[24]. Two cases of Legionnaires' disease were diagnosed in stem cell transplant patients linked to exposure to a decorative water fountain in a radiation oncology suite[25]. In a French hospital, a case of Legionnaires' disease in a leukemia patient was linked to water from a washbasin in a hematology unit[26].

3. Microbiology, Morphology, and Ecology

3.1. Taxonomy

Legionellosis, the technical name for Legionnaires' disease, is caused by bacteria known as *Legionella*. Bacteria of the family *Legionellaceae* can be found in both natural and man-made environments[27]. Some investigators have proposed placing the legionellae in three separate genera: *Legionella*, *Fluoribacter*, and *Tatlockia*[28, 29]. However, recent studies using 16S rRNA analysis confirm the family *Legionellaceae* as a single monophyletic subgroup within the gamma-2 subdivision of the Proteobacteria[30, 31]. The number of recognized species and serogroups of the genus *Legionella* continues to increase (Table 1). There are currently, the *Legionella* genus includes 52 species[32, 33]

and more than 70 different serogroups, and more than 20 species have been proven to be causative agents of Legionnaires' disease on the basis of their isolation from clinical material[34, 14]. The species *L. pneumophila* accounts for approximately 90% of confirmed cases of legionellosis, and *L. pneumophila* serogroup 1 has been recognized as the most important agent in this regard, as that specific strain was initially implicated as the pathogen causative of Legionnaires' disease in 1977[34].

3.2. Morphology

Legionellae of all types have been isolated from natural environments in different areas of the world[27]. All *Legionella* species appear as Gram-negative coccobacilli[14]. They are unencapsulated, non spore forming, with physical dimensions from 0.3 to 0.9 μm in width and from 2 to 20 μm in length[14]. Most exhibit motility through one or more polar or lateral flagella[35]. These bacteria are aerobic, microaerophilic, and have a respirative metabolism that is non-fermentative and is based on the catabolism of amino acids for energy and carbon sources[36].

3.3. Ecology

Legionella can survive in varied water conditions, in temperatures of 0-68°C, a pH range of 5.0-8.5[14], and a dissolved oxygen concentration in water of 0.2-15ppm[37]. With the exception of natural hot springs where temperature ranges from 35°C to 40°C, the sources of legionellosis are exclusively man-made water systems[38]. In water, a temperature range between 20°C to 45°C favors the growth of *L. pneumophila*[38]. At lower temperatures, *Legionella* appears to enter into a dormant stage until exposed to more favorable conditions[38]. It is found in freshwater ecosystems at low concentration, but from its natural habitat, the bacterium can colonize man made water systems where conditions are ideal for its massive growth and spreading, representing a public health problem[3]. The organisms classified in this genus are bacteria that are considered intracellular parasites. Legionellae multiplies within host cells, such as protozoa and the human macrophage[3, 39]. It's have been reported to multiply in 13 species of amoebae including *Acanthamoeba*, *Hartmannella*, *Echinamoeba*, *Tetrahymena* and *Vahlkampfia*[40] and two species of ciliated protozoa[41]. The biofilm layer, commonly associated with water-distribution systems, is considered an important niche for *Legionella*, which can survive within it. It has been hypothesized that the biofilm, in which amoebae are present, may support the survival and multiplication of legionellae, and may be even outside a host cell[3]. Biofilms may provide the necessary supply of amino acids and organic carbon that are essential to its growth[42]. In the environment, *Legionella* is also found in association within free living protozoa that it has the ability to invade and in which it can multiply[38]. The reason why *Legionella* grow inside these amoebae is because the bacteria multiply intracellularly, and therefore require the amoebae to aid in reproduction. It has

been determined that one amoebae can house enough *Legionella* bacteria to infect a human being, and this single amoebae is small enough to fit in one droplet of aerosolised water. A study evaluating factors associated with colonization of hospital water supply systems by *Legionella* spp. showed a statistically significant association between the presence of amoebae in potable water and *Legionella* colonization[43]. However, there was no correlation between concentrations of *Legionella* and the presence of amoebae[40].

4. Assessment of the Clinical Impact

The investigation of sources of sporadic cases of hospital-acquired Legionnaires' disease is often unrewarding, partly because of the ubiquitous nature of the organism. Risk assessment combined with environmental monitoring has been effective in predicting risk in studies in the USA, Italy, France, Taiwan, Spain and Greece[19, 44, 45], and most European countries now mandate routine culturing of the hospital drinking water for *Legionella*. Often, hospital water systems are colonized by *Legionella* and this contamination is responsible for most cases of hospital-acquired legionellosis. If a case of nosocomial Legionnaires' disease is detected, it is likely that additional cases will be discovered. Contamination of hospital hot water systems with *L. pneumophila* is of concern if this results in clinical illness. Some hospitals may not be aware of the occurrence of nosocomial Legionnaires' disease on their wards because of the difficulty in making the diagnosis when a routine diagnostic approach to nosocomial pneumonia is used. Approximately a quarter of all reported Legionnaires' disease cases in world acquire their infection inside a hospital. Fatality rates in nosocomial outbreaks of Legionnaires' disease can exceed 40%[46]. Hospitals caring for immunocompromised patients such as organ or bone marrow transplant recipients are at increased risk of outbreaks of Legionnaires' disease[47]. However, our understanding of the ecology and epidemiology of *Legionella* within hospital water systems[48] has improved. Other identified sources of nosocomial Legionnaires' disease that have been reported include contaminated cooling towers that were located near to a hospital ventilation air intake[49], respiratory therapy equipment that was cleaned with unsterilized tap water[50], ice machines[51] and aspiration of contaminated water associated with nasogastric feeding or swallowing disorders[52]. Legionnaires' disease is an uncommon form of pneumonia. The disease has no particular clinical features that clearly distinguish it from other types of pneumonia, and laboratory investigations must therefore be carried out in order to obtain a diagnosis. This diagnostic possibility should be pursued using appropriate diagnostic methods and a case definition. The case definitions for nosocomial Legionnaires' disease summarized[53]:

- Definite nosocomial — Legionnaires' disease in a

person who was in hospital for 10 days before the onset of symptoms.

- Probable nosocomial — Legionnaires' disease in a person who was in hospital for 1-9 of the 10 days before the onset of symptoms, and either became ill in a hospital associated with one or more previous cases of Legionnaires' disease, or yielded an isolate that was indistinguishable (by monoclonal antibody subgrouping or by molecular typing methods) from isolates obtained from the hospital water system at about the same time.

- Possible nosocomial — Legionnaires' disease in a person who was in hospital for 1-9 of the 10 days before the onset of symptoms in a hospital not previously known to be associated with any case of Legionnaires' disease, and where no microbiological link has been established between the infection and the hospital (or the residential institution).

5. Epidemiology

There is no evidence of person-to-person spread of Legionnaires' disease, infection being acquired primarily by inhalation of aerosol containing viable organisms or, particularly in immunocompromised patients, by aspiration of bacteria in water. *L. pneumophila* is the major cause of outbreaks (91.5%) and serogroup 1 (sg1) is the predominant serotype (84.2%)[55, 56]. The incidence of Legionnaires' disease depends on several factors, including the extent of contamination of the water reservoir by the organism[57, 3], susceptibility of the population exposed to that water, and the degree or intensity of the exposure of the patient to the water reservoir.

5.1. Risk Factors for Developing Nosocomial Legionnaires' Disease

The patient populations, the mode of transmission and potential environmental reservoirs are key issues when considering the likely occurrence of hospital legionellosis. Factors that render patients at higher risk of acquiring *Legionella* spp. infection comprise advanced age, underlying comorbidity, including alcoholism, diabetes, chronic obstructive pulmonary disease and Cancer (especially lung cancer or leukaemia), and cigarette smoking, and either corticosteroid or other forms of immunosuppressive therapy[57, 3]. Legionnaires' disease is considered a very rare cause of pneumonia in children immunosuppressed and all neonates were hospital-acquired[58, 14]. Surgery is a major predisposing factor in nosocomial infection, with transplant recipients at the highest risk. Transplant recipients, including renal and non-renal transplants, are highly susceptible to infection. In some series of infections in liver transplant recipients, *Legionella* spp. are among the predominant pathogens, perhaps associated with simultaneous splenectomy for associated hypersplenism[59]. AIDS patients may also be at increased risk. However, Legionnaires' disease even severe cases may occur in

previously healthy subjects and the absence of underlying disease should not be a reason to exclude the diagnosis[57]. In one series of hospitalized patients, Legionnaires' disease occurred more frequently in middle-aged men without predisposing factors, excepting that of excess alcohol intake[60].

Table 1. *Legionella* species and serogroups[54]

<i>Legionella</i> species	Serogroups	Association with clinical cases
<i>L. adlaidensis</i>		Unknown
<i>L. anisa</i>		Yes
<i>L. bleiardensis</i>		Unknown
<i>L. birminghamensis</i>		Yes
<i>L. bozemanae</i>	2	Yes
<i>L. brunensis</i>		Unknown
<i>L. busanensis</i>		Unknown
<i>L. cherrii</i>		Unknown
<i>L. cincinnatiensis</i>		Yes
<i>L. drancourtii</i>		Unknown
<i>L. dresdenensis</i>		Unknown
<i>L. drozanskii</i>		Unknown
<i>L. dumoffii</i>		Yes
<i>L. erythra</i>	2	Yes
<i>L. fairfieldensis</i>		Unknown
<i>L. fallonii</i>		Unknown
<i>L. feeleii</i>		Yes
<i>L. geestiana</i>		Unknown
<i>L. genomospecies 1</i>		Unknown
<i>L. gormanii</i>		Yes
<i>L. gratiana</i>		Unknown
<i>L. gresilensis</i>		Unknown
<i>L. hackeliae</i>	2	Yes
<i>L. israelensis</i>		Unknown
<i>L. jamestowniensis</i>		Unknown
<i>L. jordanis</i>		Yes
<i>L. lansingensis</i>		Yes
<i>L. londiniensis</i>	2	Unknown
<i>L. longbeachae</i>	2	Yes
<i>L. lytica (comb. Nov.)</i>		Unknown
<i>L. maceachernii</i>		Yes
<i>L. micdadei</i>		Yes
<i>L. moravica</i>		Unknown
<i>L. nautarum</i>		Unknown
<i>L. oakridgensis</i>		Yes
<i>L. parisiensis</i>		Yes
<i>L. pneumophila</i>	16	Yes
<i>L. quateirensis</i>		Unknown
<i>L. quinlivanii</i>	2	Unknown
<i>L. rowbothamii</i>		Unknown
<i>L. rubrilucens</i>		Unknown
<i>L. sainthelensi</i>	2	Yes
<i>L. santicrucis</i>		Unknown
<i>L. shakespearei</i>		Unknown
<i>L. spiritensis</i>	2	Unknown
<i>L. steigerwaltii</i>		Unknown
<i>L. taurinensis</i>		Unknown
<i>L. tusconensis</i>		Yes
<i>L. wadsworthii</i>		Yes
<i>L. waltersii</i>		Unknown
<i>L. worsleiensis</i>		Unknown
<i>L. yabuuchiae</i>		Unknown

5.2. Mode of transmission

The transmission of *L. pneumophila* is associated with the use of water. Legionnaires' disease can be acquired by the inhalation of droplets (1-5 μm diameter) containing aerosolized organisms[61]. Less commonly, exposure to contaminated tap water has been reported as a cause of postoperative sternal wound infection. Aerosol formation, aspiration and direct installation of bacteria in the lungs in connection with manipulation of the respiratory tract are the most common means of *Legionella* infection in hospitals environment[13]. Showering with aerosol formation is often maintained to be a frequent infection route for *Legionella* in hospitals. Surprisingly enough, some studies show that showering can prevent Legionnaires' disease[13]. The explanation given is that patients who take showers are often less ill than bed-ridden patients and therefore less at risk through aspiration. Prospective studies show that showering is not associated with nosocomial Legionnaires' disease[62]. The most important risk factor for nosocomial pneumonia is aspiration[63]. Foreign bodies that involve the oropharynx are the most important risk factors for microaspiration[63]. In patients with chronic obstructive pulmonary disease (COPD), the protective function of the airways is impaired and the disease is associated with aspiration[63]. Even a tap-water facility with a low *Legionella* level can cause the disease in immunocompromised patients[64]. Water droplets on medical equipment that are passed down into the lungs may be infected with *Legionella*[62]. Since 1982, epidemiological studies have revealed that the primary source of infection for Legionnaires' disease in hospitals is via infected water[13]. *Legionella* can be introduced into the airways by means of contaminated Puritan nebulisers. It is recommended that sterile water be used for flushing nasogastric tubes in order to prevent nosocomial Legionnaires' disease[62]. A potential mechanism by which *L. pneumophila* might be transmitted via ingestion is that of bacteremic spread after penetration of the gastrointestinal tract. Diarrhea may be a prominent symptom Legionnaires' disease. Although comparative studies have failed to conform a predilection for gastrointestinal symptoms in *L. pneumophila* as compared with pneumonias of other etiology these early clinical observations are compatible with the possibility that the gastrointestinal tract was the portal of entry. Evidence to support ingestion in humans as a mode of transmission is scanty[62].

6. Clinical manifestations

Legionella infection of man (legionellosis) can vary from a mild flu-like illness to severe life-threatening pneumonia[57]. *L. pneumophila* is the second most frequent cause of severe community acquired pneumonia after the pneumococcus. The illness has become increasingly recognized, less severely ill patients are seen earlier in the course of the disease[65]. Thus, clinical manifestations (fever, headache, diarrhea, hyponatremia) of hospital -acquired Legionnaires' disease were found to be less pronounced than for those with

community-acquired Legionnaires' disease[66]. Pneumonia is the predominant clinical syndrome. The disease presents with a broad spectrum of illness, ranging from a mild cough and low-grade fever to stupor, respiratory failure, and multiorgan failure. Early in the illness, patients have nonspecific symptoms including fever, malaise, myalgias, anorexia, and headache. The temperature often exceeds 40°C. The cough is only slightly productive. Chest pain, occasionally pleuritic, can be prominent and, when coupled with hemoptysis, may mistakenly suggest pulmonary emboli. Gastrointestinal symptoms are prominent, especially diarrhea, which occurs in 20 to 40 percent of cases. The stool is watery rather than bloody. The physical findings are those of pneumonia. The most common presentation of legionellosis is pneumonia which is often severe but almost any manifestation of the disease has been seen ranging from a mild self-limiting flu-like illness called Pontiac fever to any extrapulmonary affection to multi organ failure and death[66].

6.1. Pontiac Fever

A non pneumonic form disease associated with *Legionella* called Pontiac Fever has been described. It is an acute, self-limited illness, with "flu-like" symptoms. Numerous comparative studies of both community-acquired and nosocomial legionellosis show the clinical, radiological and laboratory features to be nonspecific[67, 57, 3]. The illness is characterized by a high attack rate of greater than 70 to 90% of exposed persons and an incubation period (typically 30 to 90 hours, with an average of 36 h)[68]. In addition, many persons who are infected with *Legionella*, as proven by seroconversion, will remain asymptomatic[69]. The illness typically resolves without complications within two to five days[68]. Upper or lower respiratory tract symptoms have not been associated with this illness. No additional information on Pontiac fever was located. Age, gender and smoking do not seem to be risk factors[68]. Rather, Pontiac Fever seems to affect preferentially young subjects: the age of cases was 36 to 39 years in the original Pontiac episode[68], and age medians during different documented epidemics were 29, 30 and 32 years[68]. Approximately 25% of cases are nosocomial in origin; the remainder is community-acquired[70]. Mortality is approximately 40% in patients with nosocomial infections and may be even higher in immunosuppressed patients[71].

6.2. Legionnaires' Disease

The clinical manifestation of Legionnaires' disease is quite varied and typically appears after 2 to 10 day incubation period[14]. There is an abrupt onset of a nonproductive cough (90%)[2], malaise, myalgia, anorexia, and headache typically occur within 48 hours. These symptoms are usually accompanied by a rapidly rising fever that frequently reaches 39°C or 40°C[72]. Chills may also occur with the fever. A dry cough is typically present in the early stages of the illness. Although the cough may become

productive with minimally or moderately purulent sputum within several days, hemoptysis is rarely observed. Other common early features of the illness include neurologic abnormalities (e.g., confusion, disorientation, lethargy) and gastrointestinal symptoms (e.g., nausea, vomiting, watery diarrhea)[2]. As the illness progresses, chest pain (often pleuritic), dyspnea, and respiratory distress may be observed. Nearly 25% of patients will exhibit some mental changes frequently confusion and disorientation. Agitation, hallucinations, depression, delirium and coma have been reported, although less frequently. Seizures, cerebellar dysfunction, and peripheral neuropathy are rare.

6.3. Extrapulmonary Diseases

Extrapulmonary diseases resulting from Legionnaires' disease are rare, but the clinical manifestations are often dramatic[73]. Since the index of suspicion is low, these infections can easily be overlooked. *Legionella* species have been implicated in cases of sinusitis[74], cellulitis[75], pancreatitis[76], brain abscess[72], acalculous cholecystitis [77] and myositis[76]. Typically, extrapulmonary infections occur concurrently with pneumonia and are believed to result from bacteremia[73]. The most common extrapulmonary site is the heart, with numerous reports of myocarditis, pericarditis postcardiotomy syndrome, and prosthetic-valve endocarditis[78]. Most such cases were acquired in the hospital. Interestingly, in many cases there was no overt pneumonia[78]; the cardiac infections may have been caused by the entry of contaminated water into postoperative sternal wound or a mediastinal tube insertion site. *Legionella* wound infections developed in several patients after cardiothoracic surgery; foreign bodies such as sutures or drainage tubes may have promoted the development of infection.

7. Pathogenicity and Virulence Factors

Legionella species are not per se adapted to the human host. That they cause disease in humans is essentially an accident of nature; the pathogenic mechanisms have evolved to enable legionellae to survive in their protozoal host also enabling them to multiply in alveolar macrophages.

7.1. Pathogenicity for Humans

Infection in human occurred following inhalation of fine aerosol containing organisms that were both viable and virulent. Once within the alveoli, the legionellae are taken up by alveolar macrophages where they multiply[79]. As a result of these multiplications in polymorphonuclear neutrophils (PMN), more alveolar macrophages and peripheral blood monocyte-macrophages[80], where the bacteria evade fusion of the phagosome to lysosome, although the mechanism maybe different in some aspects. The pathogenesis of legionellosis is largely due to the ability of *L. pneumophila* to invade and grow within alveolar macrophages[81], and it is widely believed that this ability results from a prior adaptation to intracellular niches in

nature[82]. Without lessening the importance of intracellular infection; it is likely that additional factors contribute to the survival of *L. pneumophila* in humans[83].

7.2. Properties of Potential Virulence Factors

A number of virulence factors have been described for to genes *L. pneumophila* and gene products that play a role in the infection of mammalian and protozoan cells. The study of virulence factors in such diverse hosts has led to much speculation on the evolution of intracellular pathogens. Legionellae are intracellular pathogens of macrophages, by which they are phagocytosed in a process involving the complement fragment C3 and the monocyte complement receptors CR1 and CR3[79]. Both virulent and non-virulent strains are phagocytosed, remaining intact inside the phagocytes. Virulent strains can multiply inside the phagosomes and are able to inhibit the fusion of phagosomes with lysosomes; non-virulent strains do not multiply[84]. This interaction provides an excellent selective pressure for the acquisition of factors facilitating intracellular survival and, subsequently, infection. In 1989, the first virulence associated gene of *L. pneumophila* was detected by site-specific mutagenesis[3]. This gene, designated mip for macrophage infectivity potentiator, encodes a 24-kDa surface protein (Mip)[85]. Mip is a prokaryotic homolog of the FK506-binding proteins and exhibits peptidylprolyl -cis/trans isomerase activity[86]. Subsequently, mip-like genes have been detected in other species of *Legionella* and other bacteria[3]. The exact function of *Legionella* Mip in vivo remains to be established[87]. Genes within the loci encoding the type IV secretion system of *L. pneumophila* were the first factors detected that were essential for infection of the host cell[3, 88]. These loci comprise 25 genes in two separate regions of the *Legionella* chromosome and have been named Dot/Icm (defective for organelle trafficking/intracellular multiplication)[89]. This type IV secretion system encodes factors involved in the assembly and activation of conjugal transfer of plasmid DNA[85]. *L. pneumophila* utilizes these operons to deliver virulence factors required for entering the host cell in a manner that initiates the infectious process. It is postulated that the system delivers a protein during phagocytosis that diverts the phagosome from the endocytic pathway[90]. The only secreted substrate that has been identified for the Dot/Icm system is DotA, a polytopic membrane protein[91]. Genes encoding the loci for type II secretion systems are required for unrestricted intracellular growth of *L. pneumophila*[92]. These genes were detected in *L. pneumophila* by analysis of mutants defective in type IV pilus formation[93]. The two genes that have been analyzed the most extensively are pilE (pilin protein) and pilD (prepilin peptidase)[94]. The pilE gene/pilin protein is not required for intracellular growth but may be involved in attachment to the host cell[95]. The pilD gene encodes the prepilin peptidase and is essential for pilus production and type II secretion of proteins[95].

8. Diagnosis of Legionnaires' Disease

8.1. Diagnosis Radiology and Biological

Legionnaire's disease is often classified as an "atypical pneumonia" based on the dogma that chest radiographic findings are neither lobar nor consolidating, as in the classic pyogenic pneumonias[96]. Chest radiographs typically demonstrate an initial unilateral alveolar infiltrate; however, many patterns have been reported[37]. Bilateral patterns are seen in nearly 50% of patients, and pleural effusions are detected in nearly one half of patients as well[70]. Resolution of infiltrates is frequently slow and lags behind clinical response. Leukocytosis of greater than 20,000/mm³ occurs in 10-20% of patients[70]. Hyponatremia is seen more with Legionnaires' disease than with other types of pneumonia and is most likely secondary to salt and water loss versus inappropriate antidiuretic hormone secretion. Hypophosphotemia, microhematuria, proteinuria and abnormal liver function tests are infrequent[70]. Analysis of the cerebrospinal fluid is generally within normal limits, but there are occasional reports of elevated protein or pleocytosis[37].

8.2. Bacteriological Diagnosis

Although diagnostic methods have improved since *L. pneumophila* was first described in 1976, no currently available test is able to diagnose all *Legionella* spp. in a timely fashion with a high degree of sensitivity and specificity. Most of the data are applicable to *L. pneumophila*, since sensitivity and specificity estimates for non-*pneumophila* species are not known[14]. The clinical symptoms of infection with *Legionella* are indistinguishable from the symptoms of other causes of pneumonia[38]. Accurate diagnostic methods are therefore needed to identify *Legionella*, and to provide timely and appropriate therapy. To improve diagnosis, specialized laboratory tests must be carried out, by the clinical microbiology laboratory, on patients in a high-risk category. Since 1995, diagnostic tests for legionellosis have changed significantly. The following laboratory methods are currently used for diagnosing *Legionella* infections[97]:

8.2.1. Culture

Isolation of *Legionella* spp., which has a specificity of 100%, is considered the gold standard for diagnosis of Legionnaires' disease[14]. Culture diagnosis requires special media, adequate processing of specimens, and technical expertise (Table 2). Several days are required to obtain a positive result, with most *Legionella* spp. colonies being detected within 7 days[14]. The standard medium used to culture legionellae is buffered charcoal yeast extract (BCYE) agar supplemented with α -ketoglutarate, with or without antimicrobial agents[14]. This medium provides iron and L-cysteine, both of which are essential for the growth of legionellae[98].

Table 2. Diagnostic tests for *Legionella* infection[98]

Test	Turnaround time	Sample type	Sensitivity, %	Specificity, %	Comments
Culture	3-7 Days	LTR Blood	<10-80 <10	100 100	Detects all species and serogroups Too insensitive for clinical use
Direct fluorescent antibody staining	<4 h	LRT	25-70	>95	Technically demanding
Antigen detection	<1 h	Urine	70-90	>95	Only reliable for detection of <i>Legionella pneumophila</i> serogroup 1
Serological testing	3-10 weeks	Serum	60-80	>95	Must test both acute- and convalescent-phase serum samples; single titer results can be misleading
PCR	< 4h	LRT	80-100	>90	No commercially available assay for testing clinical samples; detects all species and serogroups
		Serum	30-50	>90	-
		Urine	46-86	>90	-

The antibiotics most commonly added are polymyxin to control Gram-negative growth, anisomycin against yeasts, and cefamandole or vancomycin against Gram-positive bacteria. Vancomycin should be chosen if culture is aimed at species other than *L. pneumophila*, because cefamandole inhibits some *Legionella* spp. that does not produce beta-lactamases[14]. Legionellae can be isolated from a variety of sample types, although lower respiratory tract secretions (e.g., sputum and bronchoscopy samples) are the samples of choice. A major limitation of sputum culture is that less than one-half of patients with Legionnaires' disease produce sputum. Some patients with Legionnaires' disease produce sputum that has relatively little purulence; these samples may be rejected by laboratories that discard sputum samples containing few polymorphonuclear leukocytes[14].

8.2.2. Direct Immuno Fluorescence Assays

Direct immunofluorescence assays (DFAs) using antibody conjugated with a fluorochrome require 2-3 hours to complete the staining procedure. DFAs for *Legionella* species other than *L. pneumophila* should not ordinarily be used. DFA of sputum remains positive for 2-4 days after the initiation of the specific legionellosis antibiotic therapy, and often for a longer period in cases of a cavitary pulmonary disease[99]. Reported sensitivities of DFA staining vary, are consistently less than that of culture, and are less precisely known for species other than *L. pneumophila*. DFA has been used successfully with expectorated sputum, endotracheal suction aspirates, lung biopsies and transtracheal aspirate[97]. Pleural fluid examination in patients with legionellosis by culture or DFA rarely yields positive results, but has occasionally been helpful. Between 25% and 70% of patients with culture-proven legionellosis have positive DFA for *L. pneumophila*, and the test's specificity is higher than 99.9%. Therefore, a negative result does not rule out legionellosis but a positive result is almost always diagnostic,

provided that the slide is read correctly. Cross-reactions may be less of a problem when monoclonal-antibody DFA agents are used. Problems with sensitivity and specificity have limited the use of DFA staining, and a positive DFA result in the absence of other supporting evidence is now generally not accepted as sufficient for the diagnosis of *Legionella* infection[98].

8.2.3. Serological Testing

Serological testing for *Legionella* infection is a valuable epidemiological tool but has little impact on clinical decision making because of the time delay before a result is available. The indirect immunofluorescence assay (IFA) was used to detect antibodies in patients from the Philadelphia outbreak and was instrumental in determining the cause of the illnesses. Since then, a number of serologic test methodologies has been developed to detect antibodies to *Legionella* spp. of the various antibody detection methods that are available, Enzyme immunoassay (EIA) and microagglutination have also been used to detect antibodies to the Legionnaires' disease bacterium[3]. An indirect hemagglutination assay has been used for diagnosis of Legionnaires' disease due to *L. pneumophila* serogroups 1 to 4[3]. The rapid microagglutination test has some advantages over immunofluorescence, such as the ease of testing a large number of samples and the early appearance of agglutinating antibodies. Additional factors to consider in the serologic diagnosis of Legionnaires' disease are the use of an anti-human immunoglobulin which recognizes immunoglobulin G (IgG), IgM, and IgA[100]. These antibody responses may be serogroup specific or may react with an antigen common to *L. pneumophila*; therefore, it is not possible to reliably determine the serogroup or species causing infection[3]. Other studies have shown that, in many patients with legionellosis, the immune response is primarily IgM and that IgM tests must thus be included for optimal

sensitivity. Seroconversion may take several weeks, which is a major limitation of serological testing[14]. Approximately 25 to 40% of patients with Legionnaires' disease seroconvert within the first week after the onset of symptoms[44]. In most cases, a 4-fold increase in antibody titer is detected within 3 to 4 weeks, but in some cases, this may take more than 10 weeks. Acute-phase reciprocal IFA antibody titers of 256 in the presence of pneumonia were once considered sufficient for a presumptive diagnosis, but this has been shown to be unreliable, given the high prevalence of *Legionella* antibody positivity in persons without clinical evidence of legionellosis[4]. There is virtually no role for testing single serum samples. Another disadvantage of serological testing is the inability to accurately detect all *Legionella* species and serogroups.

Although seroconversion to *L. pneumophila* serogroup 1 is generally regarded as being highly predictive of disease, the sensitivity and specificity of seroconversion to other species and serogroups has not been rigorously confirmed. Furthermore, cross-reactive antibody formation among members of the family *Legionellaceae* can make it difficult to determine the infecting species or serogroup[98].

8.2.4. Urinary Antigen Detection

The detection of antigens from *L. pneumophila* in an infected person's urine is considered a reliable measure of the disease. An antigen excreted with urine has been characterized as heat-stable, resistant to enzymatic cleavage, and of about 10 kDa molecular weight[99]. These characteristics are typical for lipopolysaccharide components. *L. pneumophila* is now divided into at least 16 serogroups and several monoclonal subgroups[101]. *Legionella* antigenuria can be detected as early as 1 day after onset of symptoms and persists for days to weeks[98]. In one instance, excretion of antigen was documented to occur for more than 300 days[102]. The presence of antigen in the urine is a strong indicator of the disease, and a patient may have a positive response for several months following the disease. Indeed, all assays for the detection of *L. pneumophila* urinary antigens show sufficient recognition of the antigens which are homologous to the serogroup/monoclonal subgroup used as immunogen for preparation of the antisera, i.e. *L. pneumophila* sg 1[103, 104]. The sensitivity of urinary antigen detection appears to be associated with the clinical severity of disease[105]. With respect to the sensitivity for *L. pneumophila* sg 1 infections, all assays are able to detect more than 92% of cases identified by culture[103]. The major disadvantage with these tests is their inability to reliably detect organisms other than *L. pneumophila* serogroup 1[98].

8.2.5. Nucleic Acid Amplification

Over the last years, detection of nucleic acid has been more frequently used to identify *Legionella* in clinical samples. The first assay designed to detect the DNA of *L. pneumophila* was a radiolabeled ribosomal probe specific for

all strains of *Legionella* spp[3]. PCR enables specific amplification of minute amounts of *Legionella* DNA, provides results within a short time frame, and has the potential to detect infections caused by any *Legionella* species and serogroup[38]. An important feature of *Legionella* PCR is that the method can potentially detect all serogroups of *L. pneumophila* and is therefore useful in the early diagnosis of infections, particularly in nosocomial cases[106]. PCR methods could have important economic benefits. Their use in outbreaks of legionellosis could help to rapidly rule out implicated sites, thereby minimizing lost revenue and allowing resources to be diverted to areas that need further investigation. Until the diversity and distribution of legionellae are better understood, results from methods other than culture should be interpreted cautiously. PCR has been successfully used to detect *Legionella* DNA in a range of environmental and clinical samples. When testing samples from the lower respiratory tract, PCR has repeatedly been shown to have sensitivity equal to or greater than culture[107]. Indeed, PCR could be considered the test of choice for patients who produce sputum. The role of PCR for testing other sample types is less clear. *Legionella* DNA can be detected in urine, serum, and leukocyte samples obtained from patients with legionnaires disease with sensitivities of 30% to 86%[108]. The sensitivity of PCR is likely to increase when testing samples that are obtained early in the course of illness and when testing more than 1 sample type from each patient[109].

9. Prevention and Control Measures

Prevention strategies in health-care facilities in which no cases of nosocomial legionellosis have been identified have differed depending on the immunologic status of the patients, the design and construction of the facility, the resources available for implementing prevention strategies, and state and local regulations. At least two strategies are practiced with regard to the most appropriate and cost-effective means of preventing nosocomial legionellosis, especially in hospitals in which no cases or only sporadic cases of the illness have been detected.

The first approach is based on periodic, routine culturing of water samples from the hospital's potable water system for the purpose of detecting *Legionella* spp.

The second approach to preventing and controlling nosocomial legionellosis involves:

- Maintaining a high index of suspicion for legionellosis and appropriately using diagnostic tests for legionellosis in patients who have nosocomial pneumonia and who are at high risk for developing the disease and dying from the infection ;
- Initiating an investigation for a hospital source of *Legionella* spp. upon identification of one case of definite or two cases of possible nosocomial Legionnaires disease;
- Routinely maintaining cooling towers and using only sterile water for the filling and terminal rinsing of nebulizati

on devices;

The indications for a full-scale environmental investigation to search for and subsequently decontaminate identified sources of *Legionella* spp. in hospital environments have not been clarified, and these indications probably differ depending on the hospital. In hospitals in which as few as one to three nosocomial cases are identified during a period of several months, intensified surveillance for Legionnaires disease has frequently identified numerous additional cases. This finding suggests the need for a low threshold for initiating an investigation after laboratory confirmation of cases of nosocomial legionellosis. However, when developing a strategy for responding to such identification, infection-control personnel should consider the level of risk for nosocomial acquisition of, and mortality from, *Legionella* spp. infection at their particular hospital.

9.1. Assessing the Contamination of the Hot Water Distribution System

The degree of contamination with *L. pneumophila* of the hot water distribution system should be assessed in a standardized fashion. The investigation of the hot water distribution system should begin on the ward where the index case was located. The conventional plate culture method described in the standard International Organization for Standardization (ISO) 11731-2-2004 is a very important technique for the detection of *Legionella* spp. because it is the currently used standard method and it provides strains for epidemiological studies. At the moment, the standard ISO 11731-2-2004 concerning the detection and enumeration of *Legionella* spp. and *L. pneumophila* on waters is also used to detect these bacteria on sewage and sludge.

9.2. Disinfection of Water Distribution Systems for *Legionella*

Water distribution Systems are the primary reservoirs for Hospital-acquired Legionnaires' disease. Prevention of hospital-acquired Legionnaires' disease has been accomplished by disinfecting hospital water distribution systems, especially the hot water recirculating. After detection of unacceptable high levels of legionellae effective decontamination and maintenance of water are critical for prevention of outbreaks of legionellosis. In the recent years a number of methods for controlling the growth of Legionellae in drinking water supply systems: thermal (super heat and flush), hyperchlorination, copper-silver ionization, ultraviolet light sterilization, ozonation, and instantaneous steam heating systems[110]. Disinfection modalities can be classified as either focal or systemic in their application[111]. Focal disinfection refers to disinfection directed at a portion of water distribution system, usually the incoming water and the outlets. Focal disinfection approaches include ultraviolet light and instantaneous heating systems that are modular and easy to install. However, these disinfection systems are already heavily colonized with *L. pneumophila* that will persist in the biofilm throughout the water distribution system. Therefore, focal disinfection can

eliminate *Legionella* only at the point of contact[111]. Systemic disinfection refers to disinfection directed at the entire water distribution system by providing a disinfection residual that is bacteriostatic or bactericidal throughout the system, especially the distal sites and stagnant areas. These modalities include continuous hyperchlorination and copper/silver ionization. Thermal eradication (superheat and flush) is a systemic disinfection modality, however, the duration of disinfection is only short-term. Each systemic modality is highly dependent on adequate water distribution throughout the system. Failure of the disinfectant to reach the colonized area will affect the overall success of the method[111].

9.2.1. Thermal Eradication (Superheat and Flush)

Legionella species survive for months in tap[112] but are susceptible to many disinfectants, moist heat, and dry heat. The bacteria have been killed at elevated temperatures with thermal death times (D-Values) of approximately 2 minutes at 60°C and 13,9 minutes at 55°C. Raising the hot water temperature and flushing outlets was the first method successfully used for *Legionella* disinfection the "Superheat and flush" method can use in an outbreak situation, and still is used in some hospitals intermittently to suppress widespread *Legionella* contamination. The water must attain a temperature of 70°C followed by flushing of all water outlets, faucets, and showerheads with hot water for 30 minutes to kill *Legionella* colonizing these sites. Special attention must be given to the plumbing material, which must be suitable for exposure to high temperatures[111]. The disadvantages to this method are the potential for scalding and the fact that many personnel are required to monitor distal sites, tank water temperatures, flushing times, and the requirement of considerable energy[113]. In addition, recolonization will occur within months because disinfection using this method is only temporary.

9.2.2. Silver/Copper Ionisation

Copper-silver ionization systems were installed on the hot-water distribution system. This system electrolytically generates copper and silver ions, which bind to the bacterial cell wall causing cell-wall disruption and lysis[114]. Copper and Silver ions kill *L.pneumophila* in vitro[115] and in vivo[116]. The precipitation of lime on the cathode due to the production of hydroxide ions is minimised by changing the polarisation of the electrodes. Another system working on the basis of silver ions introduces a solution containing silver ions into the water proportionally to the water consumed. Silver and copper ions are removed at each water outlet by a filter. One disadvantage is that silver and copper ions are not allowed for disinfection of drinking water in each country. Silver ions may cause corrosion of the system and, furthermore, the electrodes or the silver solution have to be replaced periodically[113].

9.2.3. Ultraviolet light Irradiation

A swift local bactericidal effect is achieved with ultraviolet light (UV light) in the course of 20 minutes[117]. Ultraviolet (UV light) irradiation of the water at 254 nanometres eradicates *Legionella* without the addition of chemicals to the water. Ultraviolet light kills *Legionella* by disrupting cellular DNA synthesis[118]. It can be positioned to disinfect the incoming water, or it can be installed at a specific place in the pipe system that services a designated area. UV treatment should not be used as the sole disinfection method, because no residual protection is given in the system. Legionellae released from biofilms near water outlets are not eradicated[94]. Additionally, UV lamps are susceptible to scale and sediment deposits. The system must be installed distally of the water site. Concomitant use of bacteriological filters is recommended, which also increases the costs. UV light is not recommended in water facilities that are already contaminated with *Legionella*[117].

9.2.4. Ozone

Ozone is used in Europe for water disinfection of certain hospital services. Ozone kills *Legionella* by disrupting cellular DNA synthesis. In 1988 Domingue et al. have shown experimentally to ozone (0.1 to 0.3 mg/L) was able to eliminate 99% of *L. pneumophila* in 5 minutes[119]. Ozone is a well-known disinfectant that has been used for decades in water treatment. Low amounts of ozone are produced from aerial oxygen by corona discharge and injected into a bypass of the circulation system. A constant ozone concentration of 1-2 mg/l is required in water in order to bring about an adequate reduction in the *Legionella* concentration[117]. Ozone eradicates *Legionella* and destroys the biofilm throughout the system. Advantages of ozone include the residual protection, its decomposition to oxygen and not having to handle chemicals[113].

9.2.5. Hyperchlorination

Chlorine is an oxidizing agent that been successfully used as a disinfectant for controlling pathogens in domestic drinking water. Adding chlorine to water systems is a known method of controlling *Legionella* in both hot and cold water systems[117]. Hyperchlorination of water distribution systems requires the installation of a chlorinator. Shock chlorination is followed by continuous hyper-chlorination of the water raising chlorine levels throughout the system for one to two hours[111]. It is impossible to eradicate *Legionella* completely from the water system, and recontamination therefore takes place readily[117]. The method requires precise monitoring of the chlorine level and personnel to perform the work[117]. Chlorine can be used in the event of outbreaks or for long-term control of *Legionella*. Chlorine has no effect on *Legionella* in blind pipes, and over time it has a corrosive effect on pipe. *Legionella* forms a biofilm together with other microbes and amoebae in the pipe system. The bacteria in the biofilm are more resistant to biocides and heat treatment than freely circulating bacteria[111]. Hyperchlorination may cause human health

problems. Levels of trihalomethanes tend to increase in the hot water system when chlorine levels exceed 4mg/L[111].

10. Conclusions

Infection with *Legionella* remains an important cause of disease and death in the world. Diagnosis and treatment of legionnaires' disease should be targeted at patients at increased risk for illness and complications due to *Legionella* infection. Diagnostic tests for legionnaires' disease based on species other than *L. pneumophila*, serogroup 1, should be developed and tested. Recommendations for prevention of Legionnaires' disease should be focused on settings where there are persons at greatest risk for illness or serious outcome.

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