



Legionnaires' disease

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Lancet 2016; 387: 376–85

Published Online

July 29, 2015

[http://dx.doi.org/10.1016/S0140-6736\(15\)60078-2](http://dx.doi.org/10.1016/S0140-6736(15)60078-2)

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See Online for appendix

Since first identified in early 1977, bacteria of the genus *Legionella* are recognised as a common cause of community-acquired pneumonia and a rare cause of hospital-acquired pneumonia. *Legionella* bacteria multisystem manifestations mainly affect susceptible patients as a result of age, underlying debilitating conditions, or immunosuppression. Water is the major natural reservoir for *Legionella*, and the pathogen is found in many different natural and artificial aquatic environments such as cooling towers or water systems in buildings, including hospitals. The term given to the severe pneumonia and systemic infection caused by *Legionella* bacteria is Legionnaires' disease. Over time, the prevalence of legionellosis or Legionnaires' disease has risen, which might indicate a greater awareness and reporting of the disease. Advances in microbiology have led to a better understanding of the ecological niches and pathogenesis of the condition. Legionnaires' disease is not always suspected because of its non-specific symptoms, and the diagnostic tests routinely available do not offer the desired sensitivity. However, effective antibiotics are available. Disease notification systems provide the basis for initiating investigations and limiting the scale and recurrence of outbreaks. This report reviews our current understanding of this disease.

Introduction

Bacteria of the genus *Legionella* were discovered during the investigation of a major pneumonia outbreak in members of the American Legion attending their annual meeting in 1976 in Philadelphia.¹ The causative microorganism was an unknown bacterium and was designated *Legionella pneumophila*. The term given to the infection was Legionnaires' disease, which refers to the pneumonic form of legionellosis. 29 (16%) of 182 patients died, and this new type of pneumonia did not respond to β -lactam antibiotics. By isolating the causative bacterium, seroepidemiological studies could be done, which led to the recognition of earlier outbreaks of Legionnaires' disease. Several new serogroups of *L pneumophila* and other *Legionella* spp have since been discovered.

Since the Philadelphia outbreak, the epidemiology and pathogenesis of the disease have been clarified, and convenient non-culture-based diagnostic tests are now available. Macrolides, doxycycline, and quinolones are the main effective antibiotics, and preventive measures

have been widely adopted in public and private institutions. Here, we review the present knowledge of Legionnaires' disease and the main advances made since its identification.

Microbiology

The genus *Legionella* consists of 58 species and three subspecies. All *Legionella* bacteria have been isolated from aqueous environments and around 30 cause infection in people, mainly of the lower respiratory tract (appendix).

Legionella spp are Gram-negative bacteria with strict growth requirements.² They grow on various solid-selective and non-selective media.^{2,3} *Legionella* colonies are usually detectable after days 3–5 of incubation. Young colonies are 0.5–1 mm in diameter, self-contained, flat, smooth, with a typical ground-glass appearance and an iridescent hue. When a colony is suspected to be *Legionella*, it should be Gram stained to check for small to filamentous Gram-negative rods and plated onto two different media in the presence and absence of L-cysteine to confirm its dependence on this amino acid. The identification of *Legionella* at the species level requires more sophisticated tests than routine laboratory testing methods. These methods include: phenotypic characteristics; growth requirements; serological identification by agglutination or fluorescent antibody technique; fatty acid, carbohydrate, or ubiquinone analysis; protein profiling; and various molecular techniques.⁴

Several methods have been used to subtype *Legionella* spp.⁵ With rare exceptions, all these methods have been used to compare clinical and environmental isolates of *L pneumophila*.

Subtyping based on monoclonal antibodies directed against lipopolysaccharide epitopes on the bacterial cell surface has proved useful for *L pneumophila* serogroup 1 (Lp1) and for detecting strains expressing the virulence-associated epitope recognised by monoclonal antibodies (MAbs) 3/1 of the Dresden panel⁶ classification (mAb 2 of the International panel).⁸ This technique has also been

Search strategy and selection criteria

We searched PubMed for articles published between Jan 1, 1976, and June 31, 2014, using the MeSH terms "*Legionella*" or "Legionnaires' disease", and "history", or "epidemiology", or "microbiology", or "ecology", or "pathology or pathogenesis or pathogenicity", or "transmission", or "epidemiological monitoring or disease outbreaks", or "signs and symptoms", or "radiography", or "clinical laboratory techniques or diagnosis", or "immunocompromised host", or "therapy", or "prevention and control". These MeSH terms were also used in six secondary databases: Turning Research Into Practice (TRIP), The Cochrane Library, Dare, National Clearinghouse Guidelines, SumSearch, and National Health Service Economic Evaluations Database. There were no language restrictions. Reference textbooks were also included.^{146–151}

used to select *Legionella* strains for genotyping.^{6,7} MABs have recently been used to directly subtype urinary antigen-positive urine samples.⁹

Legionella spp are biologically inert according to traditional identification schemes. Only sequence-based schemes have the necessary resolution to confidently speciate or recognise potentially novel strains. *Legionella* bacteria are presently identified by comparing their 16S ribosomal RNA or *mip* gene sequences, with known sequences deposited in GenBank for the 16S rRNA fragment and the UK Health Protection Agency for the *mip* gene.^{10–12} A flowchart describing the identification of *Legionella* spp isolates has been published.³

Some *Legionella* spp cannot be grown on routinely used culture media and have been termed *Legionella*-like amoebal pathogens. One such pathogen was isolated from the sputum of a patient with pneumonia by amoeba enrichment.¹³ The optimum growth temperature for all but a few species of *Legionella*-like amoebal pathogens is 35°C.

Clinical manifestations

Legionnaires' disease is an atypical pneumonia that might clinically resemble pneumococcal or other bacterial pneumonias.^{14–18} Initial findings seemed to indicate a distinct clinical syndrome,¹⁹ yet some prospective studies have shown that Legionnaires' disease and pneumococcal pneumonia might have some similar clinical and radiographic findings.^{15,20–23} Symptoms range from mild disease to severe pneumonia requiring hospital admission.

The incubation period is roughly 2–14 days. A prodromal illness can occur, with symptoms such as headache, myalgia, asthenia, and anorexia. Fever is usually present except in some immunocompromised patients, and usually accompanied by relative bradycardia. The presence of gastrointestinal and neurological manifestations in patients with pneumonia should suggest Legionnaires' disease. Gastrointestinal symptoms can be prominent and include diarrhoea, nausea, vomiting, and abdominal pain. Cough produces purulent sputum in around 50% of patients, and pleuritic chest pain might occur. Headache can be prominent and accompanied by obtundation, seizures, and focal neurological findings.^{19,21,22,24,25}

From most to least common, the symptoms of Legionnaires' disease are: fever at more than 38·8°C (67–100%), cough (41–92%), chills (15–77%), dyspnoea (36–56%), fever at more than 40°C (21–62%), neurological abnormalities (38–53%), myalgia or arthralgia (20–43%), diarrhoea (19–47%), chest pain (14–50%), headache (17–43%), and nausea or vomiting (9–25%).^{19,21,22,24,25}

Recovery can be slow and patients might afterwards show fatigue, neurological and neuromuscular symptoms, and even post-traumatic stress disorder.²⁶ Non-specific laboratory findings are common, including hyponatraemia, decreased serum phosphorus, elevated

creatinine kinase, myoglobinuria, leucocytosis with relative lymphopenia, high erythrocyte sedimentation rate and C-reactive protein levels, serum ferritin elevation (more than double), and microscopic haematuria.

In most patients with Legionnaires' disease, chest radiographs show pulmonary infiltrates, although no given radiographic feature is pathognomonic. The most common pattern is a patchy, unilobar infiltrate progressing to consolidation of the lung tissue, yet all infiltrate types have been reported.^{27,28} Pleural effusion occurs in 15–50% of patients at hospital admission.²⁹ In immunosuppressed patients, especially those on glucocorticoids, round nodular opacities can appear, expanding and cavitating in about 10% of cases.³⁰ Despite appropriate antibiotic therapy, cavitation can occur up to 14 days after presentation.³¹ In a small proportion of cases, non-respiratory manifestations can occur (with or without pneumonia), such as splenomegaly and spleen rupture,³² pericarditis,³³ myocarditis,³⁴ wound infections,³⁵ endocarditis,³⁶ arthritis,³⁷ and CNS infections.^{38,39}

In patients with immunosuppression, the most common clinical presentation of Legionnaires' disease is pneumonia,⁴⁰ which may disseminate outside the lung⁴¹ or relapse,⁴² and results in a higher mortality rate than in immunocompetent hosts.¹⁹ Cavitation is also more common.

Pontiac fever is a febrile and generally benign, non-pneumonic disease associated with exposure to *Legionella* bacteria. Its pathogenesis remains obscure and there is no agreed-on definition, nor any specific clinical findings or laboratory tests for its diagnosis. Pontiac fever has been recently reported less frequently than previously, and antimicrobial treatment is usually not needed.⁴³

Epidemiology and pathogenesis

Incidence

The exact incidence of Legionnaires' disease worldwide is unknown, mainly because countries differ in awareness levels, diagnostic methods, and reporting. Legionnaires' disease accounts for 2–9% of cases of community-acquired pneumonia.⁴⁴

Data from the USA indicate a 192% increase in the crude national incidence of Legionnaires' disease, rising from 3·9 cases per million inhabitants in 2000, to 11·5 cases per million inhabitants in 2009.⁴⁵ Episodes showed seasonal variation, with 62% of cases occurring during summer and early autumn. The yearly incidence of Legionnaires' disease seem to be associated with climate changes, such as increased precipitation.⁴⁶ 24% of cases were travel-associated.⁴⁷ Only 4% of cases were associated with a known outbreak or possible cluster. The diagnosis was made by means of urinary antigen testing in 97% of cases, and only 5% were confirmed by culture.

Legionnaires' disease is substantially underdiagnosed and under-reported.⁴⁸ In 2011, 4897 cases of Legionnaires' disease were reported to the European Legionnaires' Disease Surveillance Network, with a prevalence of

9.7 cases per million inhabitants.⁴⁹ Case distribution according to place of acquisition was: community-acquired 67%; travel-associated 24%; and health-care related 7%. Only 7% of cases were reported as part of a cluster. Most cases (77%) were confirmed by the urinary antigen test.

By contrast, if thorough testing is done a very high disease prevalence is reported, for both inpatients and outpatients with community-acquired pneumonia. For example, in a multicentre study by the German Competence Network for Community-Acquired Pneumonia, which used a standardised microbiology protocol of extensive testing to diagnose *Legionella* pneumonia, the reported prevalence was 180–360 cases per million inhabitants⁵⁰ and a similar number was reported for outpatients and inpatients (3.8%). Extrapolating these data to the USA, cases of Legionnaires' disease reported to the US Centers for Disease Control and Prevention (CDC) probably represent less than 5% of actual cases.

Of all *Legionella* species, Lp1 is the most virulent and most the common cause of disease.⁴⁴ In a European-wide study of *L. pneumophila*, 1335 cases of Legionnaires' disease were serotyped; around 67% were serogroup 1 MAb 3/1-positive, and 12% were subtype MAb 3/1-negative.⁵¹ Most MAb 3/1-negative strains were isolated from nosocomial infections (53.5%); 27% from community-acquired infections and 14% from travel-associated infections.⁵¹

Only a few strains of Lp1 seem to cause most cases of Legionnaires' disease. In a US study of the prevalence of sequence types (ST) of clinical and environmental isolates of Lp1 from 1982 to 2012, ST1, ST35, ST36, ST37, and ST222 were responsible for both outbreak-associated and sporadic cases.⁵² In Europe, reported data of STs present similarities among some countries. In England and Wales, clinical and environmental isolates collected from 2000 to 2008 were subtyped as follows: 98% of clinical isolates were Lp1; 92% were MAb 3/1-positive; and ST47, ST37, and ST62 accounted for 46% of all isolates.⁵³ Of the environmental isolates, only 56% were Lp1, 8% were MAb 3/1-positive, and 34% were ST1 or ST79. There was little overlap between the two populations, and common clinical STs were rarely found in the environment. The predominant clinical STs detected in England and Wales were also identified as a cause of infection in France and the Netherlands. By contrast, the most common clinical ST in Germany was ST1. Data suggest different regions have distinct epidemiological patterns.

In patients with immunosuppression, non-*L. pneumophila* spp isolates are more common than in immunocompetent patients. After *L. pneumophila*, most *Legionella* infections in these patients are caused by *Legionella micdadei*, *Legionella bozemanii*, and *Legionella dumoffii*.

Risk factors

Risk factors for Legionnaires' disease include chronic lung disease, smoking,⁵⁴ aged older than 50 years,⁵⁵ glucocorticoid treatment,⁵⁶ haematological malignancies

under cytotoxic chemotherapy,⁵⁷ hairy cell leukaemia,⁵⁸ solid tumours,⁵⁹ and anti-tumour necrosis factor α -blocker treatment.⁶⁰ In recipients of organ transplants, Legionnaires' disease can occur any time after transplantation.^{61,62} Legionnaires' disease often coincides with rejection episodes and leads to increased morbidity or mortality.⁶³ Neutropenia has not been linked to a predisposition to *Legionella* infection.⁶⁴ Whether HIV infection is a risk factor for Legionnaires' disease is unclear.⁶⁵

Reservoir

Legionella spp are ubiquitous in aquatic habitats and water distribution systems.⁴ *L. pneumophila* withstands temperatures of 50°C for several hours yet does not multiply at temperatures below 20°C.^{4,66} The pathogens survive as intracellular parasites of amoebae, ciliated protozoa, or slime moulds.⁶⁷ Infected amoebae are found in naturally occurring microbial communities that form biofilms.⁴

Biofilm prevention is an important control measure against the proliferation of *Legionella* since, once established, it is difficult to eliminate. Factors that increase the risk of biofilm formation include the presence of nutrients (both in the source water and the materials comprising the water system), scale and corrosion, warm water temperature, and water stagnation or low flow. In low-nutrient environments, *Legionella* spp enter a slow metabolic non-replication state, which makes them difficult to recover from the environment and probably more resistant to biocides.^{40,68}

Hospital-acquired Legionnaires' disease has been linked to the presence of *Legionella* in the water supply.^{69,70} Surveys have shown that *Legionella* spp colonise hot water distribution systems in 12–70% of hospitals.⁷¹

Transmission

Legionnaires' disease is mainly transmitted via inhalation of infectious aerosols.⁷² Other less common modes are microaspiration of contaminated water or direct contact with surgical wounds.^{73,74} However, the nature of the infectious form is still unknown.⁴⁰

Many systems that produce aerosols have been linked to cases and outbreaks, including cooling towers, hot tubs, industrial equipment, domestic plumbing systems, thermal spas, water outlets, respiratory devices and nebulisers, or nasogastric tubes in hospitals.^{4,40} Cumulative exposure to the source (ie, frequency and duration of exposure and distance from the source) is a risk factor for disease acquisition.⁴⁰ The likelihood that a source will cause infection depends on the bacterial concentration, the virulence of the colonising bacteria, the effectiveness of dissemination, and the aerosol type.

For *Legionella longbeachae*, potting soil and soil conditioners containing the microorganism, not washing hands after gardening, and being close to dripping hanging flower pots have been identified as the sources of several

cases of Legionnaires' disease,⁷⁵ but the transmission mode remains unclear.^{4,76}

The causes and pathogenesis of Pontiac fever are not yet known.^{40,77} Pontiac fever is produced by inhalation of an environmental water aerosol containing microorganisms and their toxins, including *Legionella* spp.

Pathogenicity

Lp1 is the most virulent *Legionella* species and the most common cause of disease.⁴⁴ Within a single species, strains of different virulence exist, and some species and serogroups are more virulent than others.

Timothy Rowbotham first showed that *L pneumophila* could infect amoebae, and described its lifecycle in this protozoan.⁷⁸ The infection cycle starts with bacterial adhesion to host cells followed by cell entry as the most essential steps involving the flagellum, pili, and bacterial surface proteins. These proteins include the major outer membrane protein, the heat shock protein, and the mip protein.⁴ The *mip* gene was the first *L pneumophila* virulence-associated gene detected.⁶⁷ It is required for efficient host cell infection and is conserved throughout the genus.

Marcus Horwitz's experiments revealed that *L pneumophila* multiplied intracellularly in human macrophages by avoiding phagosome-lysosome fusion.⁷⁹ During phagocytosis, *Legionella* spp initiate a complex cascade of processes, including inhibition of the oxidative burst, reduced phagosome acidification, blocking of phagosome maturation, and modifications to organelle trafficking. Thus, *Legionella* spp inhibit the bactericidal activity of the phagocyte and convert the phagosome into a niche for its replication.⁴

The main virulence system in *L pneumophila* and *L longbeachae* is encoded by 26 *dot/icm* genes. This system encodes factors needed for *L pneumophila* to enter host cells, for intracellular multiplication of the pathogen, to modulate anti-apoptotic host cell signalling pathways, to disrupt and degrade the phagosome membrane, and to disrupt host cell membranes so that the bacteria egress into the extracellular environment. So far, more than 275 potential secreted effectors have been identified.

Additional virulence factors include several cytotoxins, heat shock proteins, phospholipases, lipopolysaccharides, compounds associated with iron uptake, metalloproteases, and β -lactamases. Other details about the pathogenesis of Legionnaires' disease have been described elsewhere.^{40,67,80–83}

Laboratory diagnosis

Non-culture-based methods

Legionnaires' disease can be diagnosed by both non-culture and culture techniques.

Several microscopy methods are used to detect *Legionella* spp in clinical samples. Patients with Legionnaires' disease typically produce thin watery sputum that contains few neutrophils. *Legionella* spp are small coccobacilli to short rod in shape, which are difficult to detect in clinical

samples by Gram staining.² Staining with 0.1% basic fuchsin solution rather than safranin improves imaging, but the organism is still difficult to detect.

L pneumophila can be detected by immunofluorescence microscopy of clinical samples, although the sensitivity of this procedure can be low, depending on staining quality and operator skill.⁸⁴

Urinary antigen detection is the first-line diagnostic test, although it is limited to Lp1.^{3,85} The detection of soluble antigens in urine is the fastest diagnostic technique. In Europe, the proportion of cases diagnosed using urinary antigen has significantly increased since 1995 (15% in 1995 vs over 90% in 2006). The antigen detected is a component of the cell wall lipopolysaccharide. The test is positive within 48–72 h of symptom onset and can remain positive for several weeks or months. Test sensitivity is 56–99%.^{3,86} Thus, this test could miss as many as 40% of cases of Legionnaires' disease.⁶⁷ The method is most sensitive for Lp1 MAb 3/1 subtypes, and sensitivity is lower (around 40%) for patients with Lp1 MAb 3/1-negative infection. Sensitivity correlates with disease severity.⁸⁷ Sensitivity is lower in patients with nosocomial infection or in highly immunosuppressed patients because of a greater likelihood of infections caused by *Legionella* bacteria other than *L pneumophila* or by Lp1 MAb 3/1-negative strains.⁸⁵

Some patients with Pontiac fever can also test positive for the urinary antigen. If epidemiological and clinical findings in these patients indicate Lp1-associated Pontiac fever, the test could confirm cases and the cause of an outbreak.^{77,88} Antigen detection tests need to be combined with respiratory secretion cultures so that species and serotypes not detected by the urine test can be recovered and genotyped in the event of an outbreak of Legionnaires' disease.⁸⁹

The joint guidelines⁹⁰ issued by the American Thoracic Society and the Infectious Diseases Society of America on the management of community-acquired pneumonia in adults recommend urinary antigen testing in patients not responding to outpatient antibiotic therapy, those with severe pneumonia especially if they need intensive care, immunocompromised patients, those with a history of excessive alcohol use, those who have travelled within the past 2 weeks, people aged older than 50 years, or those with pneumonia in the setting of an outbreak of Legionnaires' disease, and patients with suspected health-care-associated pneumonia.

Molecular techniques can improve diagnosis because they detect other serogroups and species and because of their higher sensitivity (about 30%) than with culture.^{12,91,92} Nucleic acid amplification-based methods have successfully identified *Legionella* spp, especially *L pneumophila*, in sputum, urine, and blood. Although six commercial assays exist, only one is approved by the US Food and Drug Administration (BD ProbeTec ET *Legionella pneumophila* amplified DNA assay) yet not marketed in the USA.^{2,93} Molecular test sensitivities of

80–100% for lower respiratory tract secretion, 30–80% for serum, and 0–90% for urine samples have been reported.^{2,91} At most laboratories, *L pneumophila* is identified through the *mip* gene.^{91,94}

High antibody titres in acute-phase serum samples are not diagnostic, since antibodies from previous subclinical *Legionella* infection could be present, as might cross-reacting antibodies from heterologous bacterial infections. In most patients with culture-confirmed Legionnaires' disease, seroconversion is not detectable until at least 3 weeks after infection, and never occurs in up to a quarter of patients with culture-proven disease. Highly immunosuppressed patients might never produce the antibodies.⁸⁴

The diagnosis of Pontiac fever involves detecting an immune response to the bacterium, although the sensitivity of this method varies and is often non-specific. Pontiac fever can only be detected with specificity by testing large groups of people with suspected disease exposed to a common source and comparing serological positivity rates with a control population.¹⁸

Sample cultures

Sample culture of the lower respiratory tract is still the gold standard for detecting Legionnaires' disease.³ Although culture can be cumbersome and technically demanding, the routine use of this technique is recommended^{2,40,89} since it enables the diagnosis of all *Legionella* spp, outbreak investigation, and further epidemiological studies, or even antimicrobial susceptibility testing. The culture of non-respiratory samples is warranted only if there is high clinical suspicion of the disease affecting other sites.² Samples for culture should be quickly transported to the laboratory in the acute infection phase, preferably before initiating antimicrobial therapy. To obtain optimum yields of *Legionella* spp, samples are diluted to limit growth inhibition by tissue and serum factors as well as antibiotics, and should be pre-treated with an acid-wash solution to minimise commensal respiratory microbiota.³ Initial isolation requires special culture media containing L-cysteine, iron, and α -ketoglutarate; and the pH should be 6.7–6.9.^{2,3,95} Buffered charcoal-yeast extract medium supplemented with 0.1% α -ketoglutaric acid is used for isolation and growth of *Legionella* spp; this medium can be made selective by adding antibiotics. Culture plates are incubated at 35°C and need high humidity. An atmosphere of 2–5% CO₂ can improve the growth of some species on solid media.⁹⁶

The sensitivity of culturing respiratory samples is 20–80% and varies with the type of sample.^{3,97,98} A low sensitivity could be attributed to patients frequently having insufficient sputum, previous antibiotic therapy, fastidious growth requirements, and expertise needed for its isolation.^{29,99} Additionally, respiratory samples are only obtained from a few patients with suspected Legionnaires' disease.^{50,91}

Infection severity affects culture yield, and patients with severe pneumonia have much higher bacterial concentrations in sputum than do those who are not as ill.⁴⁰

Antimicrobial susceptibility testing

Legionella susceptibilities to antibiotics are difficult to interpret since there is no standardised test, and in-vitro results and clinical outcomes often conflict.^{100,101} The three methods currently used are extracellular susceptibility testing (standard dilution testing in agar or broth, or E-test), in-vitro intracellular models, and animal infection models.^{100,102}

Conventional methods using broth and agar are unreliable for efficiently predicting the clinical activity of drugs. The agar used to grow *Legionella* spp binds antibiotics and reduces their activity.¹⁰³ Furthermore, the susceptibility of *L pneumophila* grown in broth or on agar might have no clinical significance, since not all antibiotics can access the bacterium because of its intracellular location.^{2,100}

In-vitro intracellular models take into account the intracellular concentrations and activities of antibiotics. Several cell models (including alveolar macrophages,¹⁰⁴ human monocytes,¹⁰⁵ and neutrophils¹⁰⁶) and tissue culture models using HeLa¹⁰⁷ or HL-60¹⁰⁸ cells, among others, have been used. Cell lines are infected with *Legionella* spp and an antibiotic is then added. The ability of the drug to inhibit intracellular growth of the bacterium is found by quantifying bacterial concentrations over time. The time needed for bacterial regrowth after drug removal is used to indicate the drug's intracellular activity. According to these observations, Edelstein classified antibiotics as non-inhibitory, reversibly inhibitory, or those that kill or cause prolonged intracellular growth inhibition after drug removal.¹⁰⁹

Generally, excellent correlation exists between susceptibility in intracellular or animal models and human disease. However, these methods are technically demanding and expensive. Additionally, the pharmacokinetics differ between animals and people, several strains or drugs cannot be tested at the same time, and these methods are used only for research purposes. Drug resistance has not been linked to treatment failure, and only one clinical isolate resistant to ciprofloxacin has been detected.¹¹⁰ Only erythromycin has been associated with treatment failures.¹¹¹

Outbreak and epidemiological surveys

When investigating an outbreak, there are several widely accepted steps:^{4,112,113} case definition, gathering epidemiological information, and testing for *Legionella* spp in the environment. A list of common environmental sites sampled for *Legionella* spp along with the approximate number and type of samples, volume of water sampled, sampling technique, transport and handling of samples, and sample processing is published elsewhere.¹¹⁴ A confirmatory epidemiological investigation is also needed.

Culture is the gold standard for the detection of *Legionella* spp in the environment.¹¹⁴ The culture method is complex, with many steps, during which substantial losses of *Legionella* spp can occur. There may also be viable but non-culturable *Legionella* not capable of multiplying on artificial media.

Non-culture methods such as quantitative PCR are more sensitive for the identification of *Legionella* in the environment,¹¹⁵ but are unable to differentiate between live and dead bacteria. Additionally, a quantitative PCR method has been developed for the rapid simultaneous detection and identification of Lp1 in both clinical and environmental samples, which has a high negative predictive value.¹¹⁵ This technique can speed up the detection and elimination of potential sources of disease.

Once *Legionella* strains are recovered, typing methods are applied to distinguish different isolates of the same species and to establish the cause of the outbreak. MAb subtyping is useful to exclude from further investigation those strains not related to clinical isolates.

Genotyping of strains isolated from patients will identify the strain that caused the outbreak, which can then be investigated in environmental sample cultures. The methods most often used are pulsed-field gel electrophoresis and amplified fragment length polymorphisms for all *Legionella* spp, and sequence-based typing for *L pneumophila*.^{5,116,117} With sequence-based typing, the strain tested is assigned to a sequence type or allele profile, which can be compared with known STs provided in the database of the European Working Group for Legionella Infections.

Typing methods have some limitations. Several studies have shown that amplified fragment length polymorphisms alone can lead to erroneous conclusions about the outbreak source.¹¹⁸ Moreover, some STs are common, which reduces effective identification when these are the cause of infection. Other STs are rare or restricted to defined local areas. Furthermore, several unrelated strains might be indistinguishable by any one method. In this situation, a combination of several methods is recommended⁵ to establish the identity or non-identity of isolates, including spoligotyping, microarrays, or whole genome sequencing.⁵ Whole genome sequencing can narrow down the possible point source of exposure, identify *Legionella* to the species level, and establish relatedness between isolates.

Prompt notification to public health authorities of strongly suspected or confirmed cases of Legionnaires' disease is essential to detect disease epidemics and is legally required in many regions.⁴⁰ WHO provides guidance on *Legionella* risk assessment and on policies and practices to be used for management of outbreaks.⁴

Environmental testing should also serve to verify the effectiveness of decontamination procedures, and is especially important in health-care facilities caring for patients at high risk of infection (ie, bone marrow or organ transplant patients).^{114,119}

Antimicrobial therapy

Legionella spp are intracellular pathogens, meaning that antibiotics against Legionnaires' disease should accumulate and be bioactive within these cells.⁴⁰ Most macrolides, tetracyclines, ketolides, and quinolones are effective.^{40,120–122} β -lactams and aminoglycosides are ineffective.

The in-vitro and intracellular activities of levofloxacin and azithromycin are similar, and are greater than those of older macrolides at inhibiting the growth of *L pneumophila*.^{123–125} However, no prospective randomised trial has compared the outcome of levofloxacin versus azithromycin.^{126,127}

Azithromycin, doxycycline, or levofloxacin can be considered, in our clinical experience, as first-line therapy. For severe or life-threatening Legionnaires' disease, the British Thoracic Society recommends the use of a fluoroquinolone.¹²⁸ Parenteral therapy is given until there is a clinical response, although outpatients with mild disease do well with oral therapy.⁹⁰

Despite the absence of supportive clinical evidence, the initial levofloxacin dose, in the opinion of some investigators, is important for optimal outcome, and the Infectious Diseases Society of America recommends a 750 mg daily dose.^{90,126,129} The standard azithromycin dose is 500 mg daily. Only anecdotes exist of the benefits of combination therapy with levofloxacin and a macrolide.¹²⁷

Recommended duration of treatment is 5–10 days for levofloxacin¹³⁰ and 3–5 days for azithromycin.^{131–133} An extended course is recommended for patients with immunosuppression, those with severe disease, empyema, and extrapulmonary infection, and those undergoing inappropriate initial therapy.¹²⁶ Early adequate therapy can reduce mortality.^{134,135}

Older macrolides sometimes interact with drugs such as tacrolimus (formerly FK-506) and ciclosporin through the cytochrome P-450 enzyme system.¹³⁶ Hence, quinolones, doxycycline, or azithromycin are preferable to treat transplant patients on these drugs.

In 2013, the US Food and Drug Administration requested the update of drug labels and medication guides for all fluoroquinolone¹³⁷ drugs to better describe their serious side-effect of peripheral neuropathy, which may be permanent, and also warned that azithromycin¹³⁸ can cause abnormal electrical heart activity. Prophylactic antibiotics have been used effectively after detection of an outbreak in high-risk populations to prevent the emergence of disease.^{135,139}

Prevention

The key to prevention of legionellosis is the proper maintenance of water systems in which *Legionella* spp grow. The water safety plan of WHO⁴ is a mechanism for implementing preventive risk management systems and should form the basis of guidelines or regulations designed to control *Legionella*. This proposal covers aspects related to drinking water quality, safe recreational water environments, ship sanitation, and health aspects of plumbing.

For the European Working Group see http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php

The 2003 US CDC guidelines for preventing health-care-associated pneumonia recommended a strategy focusing on adequate water system maintenance, universal testing of patients with nosocomial pneumonia, and investigating situations in which transmission occurs.¹⁴⁰ It also recommends the routine culturing of drinking water samples from a facility's organ-transplant unit, as well as environmental monitoring even in the absence of known cases of Legionnaires' disease.¹⁴¹

In 2000, the American Society of Heating, Refrigerating, and Air-Conditioning Engineers issued a guideline for the appropriate temperature and chemical treatment of water for legionellosis prevention in health-care facilities; this guideline is being updated.^{142,143} Copper-silver ionisation units are the favoured disinfection method but can be inadequate on their own, often needing additional bacterial suppression systems.^{144,145} Legionnaires' disease is not transmitted from person to person, so the isolation of patients admitted to hospital is unnecessary. Pontiac fever is usually described in epidemic settings and is a marker of environmental contamination by *Legionella*. Its detection should therefore prompt prevention measures to avoid an outbreak of Legionnaires' disease.

Future perspectives

Legionnaires' disease is both underdiagnosed and under-reported, so better diagnostic tests are needed for both *L. pneumophila* serogroup 1 and other serogroups and species. Such tests should be standardised and used routinely in all patients with pneumonia. Current research and risk-assessment methods are also inadequate. An improved understanding of the epidemiology of Legionnaires' disease is urgently needed to enhance the identification of environmental niches, improve risk evaluation, investigation, and control of cases and outbreaks, and to prioritise resources. Demographic changes and new risk factors in high-income countries are increasing the number of people at risk. Rapid molecular techniques are needed for a laboratory diagnosis, and novel genotyping assays with high discriminatory power to confirm environmental sources and control outbreaks are also important. Future needs are: research into *Legionella* ecology to further understand its virulence and the disease risks of the different forms of the pathogen's lifecycle and pathogenesis; the identification and assessment of the threat of *Legionella* in hot water systems along with adequate disinfection measures; improved water system maintenance; and recognising the risks of even low counts of *Legionella* in drinking water for people who are immunocompromised. Optimum therapy remains uncertain, since no adequate clinical trials have been undertaken. *L. pneumophila* has the potential to become resistant to macrolides and quinolones, although this has so far been very rare. Resistance in clinical and environmental strains should be systematically investigated, and new treatment alternatives might be needed.

Contributors

BAC, AB, and EB planned, wrote, and revised the Seminar.

Declaration of interest

We declare no competing interests.

Acknowledgments

This Seminar was partially supported by the Fondo de Investigación Sanitaria (FIS), Fund for Health Research of the Ministry of Health and Consumer Services of Spain (PSI 09/01257). The funder of this Seminar had no role in design, data collection, data analysis, data interpretation, or writing of the report.

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