Legionnaires' disease

Burke A Cunha, Almudena Burillo, Emilio Bouza

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.

Published Online
July 29, 2015
http://dx.doi.org/10.1016/S0140-6736(15)60078-2

Infectious Disease Division, Winthrop-University Hospital, Mineola, NY, USA (Prof B A Cunha MD)**; School of Medicine, State University of New York, Stony Brook, NY, USA** (Prof B A Cunha)**; Division of Clinical Microbiology and Infectious Disease, Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain** (A Burillo MD, Prof E Bouza MD)**; Universidad Complutense de Madrid, Madrid, Spain** (A Burillo, Prof E Bouza)**; and CIBER de Enfermedades Respiratorias (CIBERES CB06/06/0058), Madrid, Spain** (Prof E Bouza)

Correspondence to:
Prof Burke A Cunha, Infectious Disease Division, Winthrop-University Hospital, 222 Station Plaza North, Mineola, NY 11501, USA
bacunha@winthrop.org

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 aminoacid. 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 panel[6] classification (mAb 2 of the International panel[7]).[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]

See Online for appendix

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 creatine 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 lung[41] 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 inhabitants[50] 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 bozemanae*, 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 guidelines[90] 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% CO_2 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 neutrophils[106]) and tissue culture models using HeLa[107] or HL-60[108] 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,[113] 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.

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

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 recommended[5] 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 levofloxacin[130] 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 fluoroquinolone[137] drugs to better describe their serious side-effect of peripheral neuropathy, which may be permanent, and also warned that azithromycin[138] 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 WHO[4] 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.

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.

References

1 Fraser DW, Tsai TR, Orenstein W, et al. Legionnaires' disease: description of an epidemic of pneumonia. *N Engl J Med* 1977; **297:** 1189–97.

2 Edelstein PH. *Legionella*. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, eds. Manual of clinical microbiology, 10th edn. Washington, DC: American Society of Microbiology Press, 2011: 770–85.

3 Jarraud S, Descours G, Ginevra C, Lina G, Etienne J. Identification of legionella in clinical samples. *Methods Mol Biol* 2013; **954:** 27–56.

4 Bartram J, Chartier Y, Lee JV, Pond K, Surman-Lee S. *Legionella* and the prevention of legionellosis. Geneva, Switzerland: World Health Organization Press, 2007. http://www.who.int/water_sanitation_health/emerging/legionella.pdf (accessed June 1, 2014).

5 Lück C, Fry NK, Helbig JH, Jarraud S, Harrison TG. Typing methods for legionella. *Methods Mol Biol* 2013; **954:** 119–48.

6 Joly JR, McKinney RM, Tobin JO, Bibb WF, Watkins ID, Ramsay D. Development of a standardized subgrouping scheme for *Legionella pneumophila* serogroup 1 using monoclonal antibodies. *J Clin Microbiol* 1986; **23:** 768–71.

7 Helbig JH, Kurtz JB, Pastoris MC, Pelaz C, Lück PC. Antigenic lipopolysaccharide components of *Legionella pneumophila* recognized by monoclonal antibodies: possibilities and limitations for division of the species into serogroups. *J Clin Microbiol* 1997; **35:** 2841–45.

8 Dournon E, Bibb WF, Rajagopalan P, Desplaces N, McKinney RM. Monoclonal antibody reactivity as a virulence marker for *Legionella pneumophila* serogroup 1 strains. *J Infect Dis* 1988; **157:** 496–501.

9 Helbig JH, Jacobs E, Lück C. *Legionella pneumophila* urinary antigen subtyping using monoclonal antibodies as a tool for epidemiological investigations. *Eur J Clin Microbiol Infect Dis* 2012; **31:** 1673–77.

10 Fry NK, Warwick S, Saunders NA, Embley TM. The use of 16S ribosomal RNA analyses to investigate the phylogeny of the family *Legionellaceae*. *J Gen Microbiol* 1991; **137:** 1215–22.

11 Ratcliff RM, Lanser JA, Manning PA, Heuzenroeder MW. Sequence-based classification scheme for the genus *Legionella* targeting the mip gene. *J Clin Microbiol* 1998; **36:** 1560–67.

12 Ratcliff RM. Sequence-based identification of legionella. *Methods Mol Biol* 2013; **954:** 57–72.

13 Fry NK, Rowbotham TJ, Saunders NA, Embley TM. Direct amplification and sequencing of the 16S ribosomal DNA of an intracellular *Legionella* species recovered by amoebal enrichment from the sputum of a patient with pneumonia. *FEMS Microbiol Lett* 1991; **67:** 165–68.

14 Cunha BA. Clinical features of legionnaires' disease. *Semin Respir Infect* 1998; **13:** 116–27.

15 Mulazimoglu L, Yu VL. Can Legionnaires disease be diagnosed by clinical criteria? A critical review. *Chest* 2001; **120:** 1049–53.

16 Cunha BA. Severe *Legionella* pneumonia: rapid presumptive clinical diagnosis with Winthrop-University Hospital's weighted point score system (modified). *Heart Lung* 2008; **37:** 311–20.

17 Cunha BA. Legionnaires' disease: clinical differentiation from typical and other atypical pneumonias. *Infect Dis Clin North Am* 2010; **24:** 73–105.

18 Edelstein PH. Legionnaires' disease: history and clinical findings. In: Heuner K, Swanson M, eds. *Legionella* molecular microbiology, 1st edn. Norfolk, UK: Caister Academic Press, 2008: 1–18.

19 Kirby BD, Snyder KM, Meyer RD, Finegold SM. Legionnaires' disease: report of sixty-five nosocomially acquired cases of review of the literature. *Medicine (Baltimore)* 1980; **59:** 188–205.

20 Woodhead MA, Macfarlane JT. Comparative clinical and laboratory features of legionella with pneumococcal and mycoplasma pneumonias. *Br J Dis Chest* 1987; **81:** 133–39.

21 Granados A, Podzamczer D, Gudiol F, Manresa F. Pneumonia due to *Legionella pneumophila* and pneumococcal pneumonia: similarities and differences on presentation. *Eur Respir J* 1989; **2:** 130–34.

22 Roig J, Aguilar X, Ruiz J, et al. Comparative study of *Legionella pneumophila* and other nosocomial-acquired pneumonias. *Chest* 1991; **99:** 344–50.

23 Gupta SK, Imperiale TF, Sarosi GA. Evaluation of the Winthrop-University Hospital criteria to identify *Legionella* pneumonia. *Chest* 2001; **120:** 1064–71.

24 Yu VL, Kroboth FJ, Shonnard J, Brown A, McDearman S, Magnussen M. Legionnaires' disease: new clinical perspective from a prospective pneumonia study. *Am J Med* 1982; **73:** 357–61.

25 Fang GD, Fine M, Orloff J, et al. New and emerging etiologies for community-acquired pneumonia with implications for therapy. A prospective multicenter study of 359 cases. *Medicine (Baltimore)* 1990; **69:** 307–16.

26 Lettinga KD, Verbon A, Nieuwkerk PT, et al. Health-related quality of life and posttraumatic stress disorder among survivors of an outbreak of Legionnaires disease. *Clin Infect Dis* 2002; **35:** 11–17.

27 Tan MJ, Tan JS, Hamor RH, File TM Jr, Breiman RF, and The Ohio Community-Based Pneumonia Incidence Study Group. The radiologic manifestations of Legionnaire's disease. *Chest* 2000; **117:** 398–403.

28 Cunha BA, Pherez FM, Nouri Y. *Legionella* community-acquired pneumonia (CAP) presenting with spontaneous bilateral pneumothoraces. *Heart Lung* 2008; **37:** 238–41.

29 Viasus D, Di Yacovo S, Garcia-Vidal C, et al. Community-acquired *Legionella pneumophila* pneumonia: a single-center experience with 214 hospitalized sporadic cases over 15 years. *Medicine (Baltimore)* 2013; **92:** 51–60.

30 Yu H, Higa F, Koide M, et al. Lung abscess caused by *Legionella* species: implication of the immune status of hosts. *Intern Med* 2009; **48:** 1997–2002.

31 Bouza Santiago E, Rodríguez Creixems M. Legionnaires' disease. Review. *Med Clin (Barc)* 1981; **77:** 298–310 (in Spanish).

32 Saura P, Vallés J, Jubert P, Ormaza J, Segura F. Spontaneous rupture of the spleen in a patient with legionellosis. *Clin Infect Dis* 1993; **17:** 298.

33 Mayock R, Skale B, Kohler RB. *Legionella pneumophila* pericarditis proved by culture of pericardial fluid. *Am J Med* 1983; **75:** 534–36.

34 Armengol S, Domingo C, Mesalles E. Myocarditis: a rare complication during *Legionella* infection. *Int J Cardiol* 1992; **37:** 418–20.

35 Brabender W, Hinthorn DR, Asher M, Lindsey NJ, Liu C. *Legionella pneumophila* wound infection. *JAMA* 1983; **250:** 3091–92.

36 Menasalvas A, Bouza E. Infective endocarditis caused by unusual microorganisms. *Rev Esp Cardiol* 1998; **51** (suppl 2)**:** 79–85 (in Spanish).

37 Fernández-Cruz A, Marín M, Castelo L, Usubillaga R, Martín-Rabadán P, Bouza E, and the GAIO (Group for the Assessment of Osteoarticular Infections) Study Group. *Legionella micdadei*, a new cause of prosthetic joint infection. *J Clin Microbiol* 2011; **49:** 3409–10.

38 Heath PD, Booth L, Leigh PN, Turner AM. *Legionella* brain stem encephalopathy and peripheral neuropathy without preceding pneumonia. *J Neurol Neurosurg Psychiatry* 1986; **49:** 216–18.

39 Andersen BB, Søgaard I. Legionnaires' disease and brain abscess. *Neurology* 1987; **37:** 333–34.

40 Edelstein PH, Cianciotto NP. *Legionella*. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett's principles and practice of infectious diseases, 7th edn. Philadelphia: Churchill Livingstone Elsevier, 2010: 2969–84.

41 Weisenburger DD, Helms CM, Renner ED. Sporadic Legionnaires' disease. A pathologic study of 23 fatal cases. *Arch Pathol Lab Med* 1981; **105:** 130–37.

42 van't Hullenaar NG, van Ketel RJ, Kuijper EJ, Bakker PJ, Dankert J. Relapse of *Legionella longbeachae* infection in an immunocompromised patient. *Neth J Med* 1996; **49:** 202–04.

43 Cordes LG, Fraser DW. Legionellosis: Legionnaires' disease; Pontiac fever. *Med Clin North Am* 1980; **64:** 395–416.

44 Stout JE, Yu VL. Legionellosis. *N Engl J Med* 1997; **337:** 682–87.

45 Centers for Disease Control and Prevention (CDC). Legionellosis—United States, 2000–2009. *MMWR Morb Mortal Wkly Rep* 2011; **60:** 1083–86.

46 Cunha BA, Connolly J, Abruzzo E. Increase in pre-seasonal community-acquired Legionnaire's disease due to increased precipitation. *Clin Microbiol Infect* 2015; **21:** e45–46.

47 Garcia-Vidal C, Labori M, Viasus D, et al. Rainfall is a risk factor for sporadic cases of *Legionella pneumophila* pneumonia. *PLoS One* 2013; **8:** e61036.

48 Garrison LE, Shaw KM, McCollum JT, et al. On-site availability of *Legionella* testing in acute care hospitals, United States. *Infect Control Hosp Epidemiol* 2014; **35:** 898–900.

49 European Centre for Disease Prevention and Control. Legionnaires' disease in Europe, 2011. Stockholm: ECDC, 2013. http://ecdc.europa.eu/en/publications/Publications/legionnaires-disease-in-europe-2011.pdf (accessed June 1, 2014).

50 von Baum H, Ewig S, Marre R, et al, and the Competence Network for Community Acquired Pneumonia Study Group. Community-acquired *Legionella* pneumonia: new insights from the German competence network for community acquired pneumonia. *Clin Infect Dis* 2008; **46:** 1356–64.

51 Helbig JH, Bernander S, Castellani Pastoris M, et al. Pan-European study on culture-proven Legionnaires' disease: distribution of *Legionella pneumophila* serogroups and monoclonal subgroups. *Eur J Clin Microbiol Infect Dis* 2002; **21:** 710–16.

52 Kozak-Muiznieks NA, Lucas CE, Brown E, et al. Prevalence of sequence types among clinical and environmental isolates of *Legionella pneumophila* serogroup 1 in the United States from 1982 to 2012. *J Clin Microbiol* 2014; **52:** 201–11.

53 Harrison TG, Afshar B, Doshi N, Fry NK, Lee JV. Distribution of *Legionella pneumophila* serogroups, monoclonal antibody subgroups and DNA sequence types in recent clinical and environmental isolates from England and Wales (2000–2008). *Eur J Clin Microbiol Infect Dis* 2009; **28:** 781–91.

54 Doebbeling BN, Wenzel RP. The epidemiology of *Legionella pneumophila* infections. *Semin Respir Infect* 1987; **2:** 206–21.

55 England AC 3rd, Fraser DW, Plikaytis BD, Tsai TF, Storch G, Broome CV. Sporadic legionellosis in the United States: the first thousand cases. *Ann Intern Med* 1981; **94:** 164–70.

56 Hofflin JM, Potasman I, Baldwin JC, Oyer PE, Stinson EB, Remington JS. Infectious complications in heart transplant recipients receiving cyclosporine and corticosteroids. *Ann Intern Med* 1987; **106:** 209–16.

57 Kugler JW, Armitage JO, Helms CM, et al. Nosocomial Legionnaires' disease. Occurrence in recipients of bone marrow transplants. *Am J Med* 1983; **74:** 281–88.

58 Radaelli F, Langer M, Chiorboli O, Proietti D, Baldini L. Severe *Legionella pneumophila* infection in a patient with hairy cell leukemia in partial remission after alpha interferon treatment. *Hematol Oncol* 1991; **9:** 125–28.

59 Marston BJ, Lipman HB, Breiman RF. Surveillance for Legionnaires' disease. Risk factors for morbidity and mortality. *Arch Intern Med* 1994; **154:** 2417–22.

60 Lanternier F, Tubach F, Ravaud P, et al, and the Research Axed on Tolerance of Biotherapies Group. Incidence and risk factors of *Legionella pneumophila* pneumonia during anti-tumor necrosis factor therapy: a prospective French study. *Chest* 2013; **144:** 990–98.

61 Myerowitz RL, Pasculle AW, Dowling JN, et al. Opportunistic lung infection due to "Pittsburgh Pneumonia Agent". *N Engl J Med* 1979; **301:** 953–58.

62 Singh N, Stout JE, Yu VL. Prevention of Legionnaires' disease in transplant recipients: recommendations for a standardized approach. *Transpl Infect Dis* 2004; **6:** 58–62.

63 Ampel NM, Wing EJ. *Legionella* infection in transplant patients. *Semin Respir Infect* 1990; **5:** 30–37.

64 Yu VL. *Legionella pneumophila* (Legionnaires' Disease). In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, 7th edn. Philadelphia: Churchill Livingstone Elsevier, 2000: 2424–35.

65 Sandkovsky U, Sandkovsky G, Suh J, Smith B, Sharp V, Polsky B. *Legionella* pneumonia and HIV: case reports and review of the literature. *AIDS Patient Care STDS* 2008; **22:** 473–81.

66 Fliermans CB, Soracco RJ, Pope DH. Measure of *Legionella pneumophila* activity in situ. *Curr Microbiol* 1981; **6:** 89–94.

67 Fields BS, Benson RF, Besser RE. *Legionella* and Legionnaires' disease: 25 years of investigation. *Clin Microbiol Rev* 2002; **15:** 506–26.

68 Steinert M, Emödy L, Amann R, Hacker J. Resuscitation of viable but nonculturable *Legionella pneumophila* Philadelphia JR32 by *Acanthamoeba castellanii*. *Appl Environ Microbiol* 1997; **63:** 2047–53.

69 Stout J, Yu VL, Vickers RM, et al. Ubiquitousness of *Legionella pneumophila* in the water supply of a hospital with endemic Legionnaires' disease. *N Engl J Med* 1982; **306:** 466–68.

70 Allen JG, Myatt TA, Macintosh DL, et al. Assessing risk of health care-acquired Legionnaires' disease from environmental sampling: the limits of using a strict percent positivity approach. *Am J Infect Control* 2012; **40:** 917–21.

71 Lin YS, Stout JE, Yu VL, Vidic RD. Disinfection of water distribution systems for *Legionella*. *Semin Respir Infect* 1998; **13:** 147–59.

72 Fraser DW. Legionellosis: evidence of airborne transmission. *Ann N Y Acad Sci* 1980; **353:** 61–66.

73 Johnson JT, Yu VL, Best MG, et al. Nosocomial legionellosis in surgical patients with head-and-neck cancer: implications for epidemiological reservoir and mode of transmission. *Lancet* 1985; **2:** 298–300.

74 Marrie TJ, Haldane D, MacDonald S, et al. Control of endemic nosocomial legionnaires' disease by using sterile potable water for high risk patients. *Epidemiol Infect* 1991; **107:** 591–605.

75 Steele TW, Lanser J, Sangster N. Isolation of *Legionella longbeachae* serogroup 1 from potting mixes. *Appl Environ Microbiol* 1990; **56:** 49–53.

76 O'Connor BA, Carman J, Eckert K, Tucker G, Givney R, Cameron S. Does using potting mix make you sick? Results from a *Legionella longbeachae* case-control study in South Australia. *Epidemiol Infect* 2007; **135:** 34–39.

77 Edelstein PH. Urine antigen tests positive for Pontiac fever: implications for diagnosis and pathogenesis. *Clin Infect Dis* 2007; **44:** 229–31.

78 Rowbotham TJ. Preliminary report on the pathogenicity of *Legionella pneumophila* for freshwater and soil amoebae. *J Clin Pathol* 1980; **33:** 1179–83.

79 Horwitz MA. Formation of a novel phagosome by the Legionnaires' disease bacterium (*Legionella pneumophila*) in human monocytes. *J Exp Med* 1983; **158:** 1319–31.

80 Pasculle AW. Pathogenesis and pathology of legionellosis. *Semin Respir Infect* 1987; **2:** 222–27.

81 Dowling JN, Saha AK, Glew RH. Virulence factors of the family *Legionellaceae*. *Microbiol Rev* 1992; **56:** 32–60.

82 Newton HJ, Ang DK, van Driel IR, Hartland EL. Molecular pathogenesis of infections caused by *Legionella pneumophila*. *Clin Microbiol Rev* 2010; **23:** 274–98.

83 Hilbi H, Jarraud S, Hartland E, Buchrieser C. Update on Legionnaires' disease: pathogenesis, epidemiology, detection and control. *Mol Microbiol* 2010; **76:** 1–11.

84 Edelstein PH, Meyer RD, Finegold SM. Laboratory diagnosis of Legionnaires' disease. *Am Rev Respir Dis* 1980; **121:** 317–27.

85 Helbig JH, Uldum SA, Bernander S, et al. Clinical utility of urinary antigen detection for diagnosis of community-acquired, travel-associated, and nosocomial legionnaires' disease. *J Clin Microbiol* 2003; **41:** 838–40.

86 Shimada T, Noguchi Y, Jackson JL, et al. Systematic review and metaanalysis: urinary antigen tests for Legionellosis. *Chest* 2009; **136:** 1576–85.

87 Yzerman EP, den Boer JW, Lettinga KD, Schellekens J, Dankert J, Peeters M. Sensitivity of three urinary antigen tests associated with clinical severity in a large outbreak of Legionnaires' disease in The Netherlands. *J Clin Microbiol* 2002; **40:** 3232–36.

88 Burnsed LJ, Hicks LA, Smithee LM, et al, and the Legionellosis Outbreak Investigation Team. A large, travel-associated outbreak of legionellosis among hotel guests: utility of the urine antigen assay in confirming Pontiac fever. *Clin Infect Dis* 2007; **44:** 222–28.

89 Pasculle AW, McDevitt D. *Legionella* cultures. In: Garcia LS, ed. Clinical microbiology procedures handbook, 3rd edn. Washington, DC: American Society for Microbiology Press, 2010.

90 Mandell LA, Wunderink RG, Anzueto A, et al, and the Infectious Diseases Society of America, and the American Thoracic Society. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2007; **44** (suppl 2): S27–72.

91 Mentasti M, Fry NK, Afshar B, Palepou-Foxley C, Naik FC, Harrison TG. Application of *Legionella pneumophila*-specific quantitative real-time PCR combined with direct amplification and sequence-based typing in the diagnosis and epidemiological investigation of Legionnaires' disease. *Eur J Clin Microbiol Infect Dis* 2012; **31:** 2017–28.

92 Murdoch DR, Podmore RG, Anderson TP, et al. Impact of routine systematic polymerase chain reaction testing on case finding for Legionnaires' disease: a pre-post comparison study. *Clin Infect Dis* 2013; **57:** 1275–81.

93 Cho MC, Kim H, An D, et al. Comparison of sputum and nasopharyngeal swab specimens for molecular diagnosis of *Mycoplasma pneumoniae, Chlamydophila pneumoniae*, and *Legionella pneumophila*. *Ann Lab Med* 2012; **32:** 133–38.

94 Nazarian EJ, Bopp DJ, Saylors A, Limberger RJ, Musser KA. Design and implementation of a protocol for the detection of *Legionella* in clinical and environmental samples. *Diagn Microbiol Infect Dis* 2008; **62:** 125–32.

95 Chatfield CH, Cianciotto NP. Culturing, media, and handling of legionella. *Methods Mol Biol* 2013; **954:** 151–62.

96 Winn WC Jr. *Legionella* and the clinical microbiologist. *Infect Dis Clin North Am* 1993; **7:** 377–92.

97 Breiman RF, Butler JC. Legionnaires' disease: clinical, epidemiological, and public health perspectives. *Semin Respir Infect* 1998; **13:** 84–89.

98 Cloud JL, Carroll KC, Pixton P, Erali M, Hillyard DR. Detection of *Legionella* species in respiratory specimens using PCR with sequencing confirmation. *J Clin Microbiol* 2000; **38:** 1709–12.

99 Yu VL, Stout JE. Community-acquired legionnaires disease: implications for underdiagnosis and laboratory testing. *Clin Infect Dis* 2008; **46:** 1365–67.

100 Edelstein PH. Antimicrobial chemotherapy for legionnaires' disease: a review. *Clin Infect Dis* 1995; **21** (suppl 3): S265–76.

101 Sabria M, Pedro-Botet ML, Yu V, Lin YE. *Legionella* species (Legionnaires' disease) In: Yu V, Burdette SD, eds. Manual of Infectious Diseases; 2013. http://www.antimicrobe.org/b118rev.asp#9 (accessed June 1, 2014).

102 Rhomberg PR, Bale MJ, Jones RN. Application of the Etest to antimicrobial susceptibility testing of *Legionella* spp. *Diagn Microbiol Infect Dis* 1994; **19:** 175–78.

103 Pohlod DJ, Saravolatz LD, Quinn EL, Somerville MM. The effect of inoculum, culture medium and antimicrobial combinations on the in-vitro susceptibilities of *Legionella pneumophila*. *J Antimicrob Chemother* 1981; **7:** 335–41.

104 Edelstein PH, Edelstein MA. In vitro activity of azithromycin against clinical isolates of *Legionella* species. *Antimicrob Agents Chemother* 1991; **35:** 180–81.

105 Vildé JL, Dournon E, Rajagopalan P. Inhibition of *Legionella pneumophila* multiplication within human macrophages by antimicrobial agents. *Antimicrob Agents Chemother* 1986; **30:** 743–48.

106 Anderson R, Joone G, van Rensburg CE. An in-vitro evaluation of the cellular uptake and intraphagocytic bioactivity of clarithromycin (A-56268, TE-031), a new macrolide antimicrobial agent. *J Antimicrob Chemother* 1988; **22:** 923–33.

107 Goldoni P, Castellani Pastoris M, et al. Effect of monensin on the invasiveness and multiplication of *Legionella pneumophila*. *J Med Microbiol* 1995; **42:** 269–75.

108 Stout JE, Arnold B, Yu VL. Comparative activity of ciprofloxacin, ofloxacin, levofloxacin, and erythromycin against *Legionella* species by broth microdilution and intracellular susceptibility testing in HL-60 cells. *Diagn Microbiol Infect Dis* 1998; **30:** 37–43.

109 Edelstein PH, Edelstein MA. In vitro extracellular and intracellular activities of clavulanic acid and those of piperacillin and ceftriaxone alone and in combination with tazobactam against clinical isolates of *Legionella* species. *Antimicrob Agents Chemother* 1994; **38:** 200–04.

110 Bruin JP, Koshkolda T, IJzerman EP, et al. Isolation of ciprofloxacin-resistant *Legionella pneumophila* in a patient with severe pneumonia. *J Antimicrob Chemother* 2014; **69:** 2869–71.

111 Klein NC, Cunha BA. Treatment of Legionnaire's disease. *Semin Respir Infect* 1998; **13:** 140–46.

112 Joseph C. Investigation of outbreaks: epidemiology. *Methods Mol Biol* 2013; **954:** 73–86.

113 Lee S, Lee J. Outbreak investigations and identification of legionella in contaminated water. *Methods Mol Biol* 2013; **954:** 87–118.

114 Kozak NA, Lucas CE, Winchell JM. Identification of legionella in the environment. *Methods Mol Biol* 2013; **954:** 3–25.

115 Mérault N, Rusniok C, Jarraud S, et al, and the DELPH-I Study Group. Specific real-time PCR for simultaneous detection and identification of *Legionella pneumophila* serogroup 1 in water and clinical samples. *Appl Environ Microbiol* 2011; **77:** 1708–17.

116 Gaia V, Fry NK, Afshar B, et al. Consensus sequence-based scheme for epidemiological typing of clinical and environmental isolates of *Legionella pneumophila*. *J Clin Microbiol* 2005; **43:** 2047–52.

117 Ratzow S, Gaia V, Helbig JH, Fry NK, Lück PC. Addition of neuA, the gene encoding N-acylneuraminate cytidylyl transferase, increases the discriminatory ability of the consensus sequence-based scheme for typing *Legionella pneumophila* serogroup 1 strains. *J Clin Microbiol* 2007; **45:** 1965–68.

118 Bernander S, Jacobson K, Lundholm M. A hospital-associated outbreak of Legionnaires' disease caused by *Legionella pneumophila* serogroups 4 and 10 with a common genetic fingerprinting pattern. *APMIS* 2004; **112:** 210–17.

119 Lin YE, Stout JE, Yu VL. Prevention of hospital-acquired legionellosis. *Curr Opin Infect Dis* 2011; **24:** 350–56.

120 Dunbar LM, Khashab MM, Kahn JB, Zadeikis N, Xiang JX, Tennenberg AM. Efficacy of 750-mg, 5-day levofloxacin in the treatment of community-acquired pneumonia caused by atypical pathogens. *Curr Med Res Opin* 2004; **20:** 555–63.

121 Garau J, Fritsch A, Arvis P, Read RC. Clinical efficacy of moxifloxacin versus comparator therapies for community-acquired pneumonia caused by *Legionella* spp. *J Chemother* 2010; **22:** 264–66.

122 Bruin JP, Ijzerman EP, den Boer JW, Mouton JW, Diederen BM. Wild-type MIC distribution and epidemiological cut-off values in clinical *Legionella pneumophila* serogroup 1 isolates. *Diagn Microbiol Infect Dis* 2012; **72:** 103–08.

123 Edelstein PH, Hu B, Edelstein MA. In vitro and intracellular activities of LBM415 (NVP PDF-713) against *Legionella pneumophila*. *Antimicrob Agents Chemother* 2005; **49:** 2533–35.

124 Erdogan H, Can F, Demirbilek M, Timurkaynak F, Arslan H. In vitro activity of antimicrobial agents against *Legionella* isolated from environmental water systems: first results from Turkey. *Environ Monit Assess* 2010; **171:** 487–91.

125 Sato T, Tateda K, Kimura S, Ishii Y, Yamaguchi K. In vitro intracellular activity and in vivo efficacy of modithromycin, a novel bicyclolide, against *Legionella pneumophila*. *Antimicrob Agents Chemother* 2011; **55:** 1594–97.

126 Pedro-Botet ML, Yu VL. Treatment strategies for *Legionella* infection. *Expert Opin Pharmacother* 2009; **10:** 1109–21.

127 Carratalà J, Garcia-Vidal C. An update on *Legionella*. *Curr Opin Infect Dis* 2010; **23:** 152–57.

128 Levy ML, Le Jeune I, Woodhead MA, Macfarlaned JT, Lim WS, and the British Thoracic Society Community Acquired Pneumonia in Adults Guideline Group. Primary care summary of the British Thoracic Society Guidelines for the management of community acquired pneumonia in adults: 2009 update. Endorsed by the Royal College of General Practitioners and the Primary Care Respiratory Society UK. *Prim Care Respir J* 2010; **19:** 21–27.

129 Martinez MN, Papich MG, Drusano GL. Dosing regimen matters: the importance of early intervention and rapid attainment of the pharmacokinetic/pharmacodynamic target. *Antimicrob Agents Chemother* 2012; **56:** 2795–805.

130 Yu VL, Greenberg RN, Zadeikis N, et al. Levofloxacin efficacy in the treatment of community-acquired legionellosis. *Chest* 2004; **125:** 2135–39.

131 Kuzman I, Soldo I, Schönwald S, Culig J. Azithromycin for treatment of community acquired pneumonia caused by *Legionella pneumophila*: a retrospective study. *Scand J Infect Dis* 1995; **27:** 503–05.

132 Sánchez F, Mensa J, Martínez JA, et al. Treatment of pneumonia caused by Legionella with azithromycin. *Rev Esp Quimioter* 1998; **11:** 147–51 (in Spanish).

133 Edelstein PH. Antimicrobial chemotherapy for Legionnaires disease: time for a change. *Ann Intern Med* 1998; **129:** 328–30.

134 Heath CH, Grove DI, Looke DF. Delay in appropriate therapy of *Legionella* pneumonia associated with increased mortality. *Eur J Clin Microbiol Infect Dis* 1996; **15:** 286–90.

135 Gudiol C, Verdaguer R, Angeles Domínguez M, Fernández-Sevilla A, Carratalà J. Outbreak of Legionnaires' disease in immunosuppressed patients at a cancer centre: usefulness of universal urine antigen testing and early levofloxacin therapy. *Clin Microbiol Infect* 2007; **13:** 1125–28.

136 Paterson DL, Singh N. Interactions between tacrolimus and antimicrobial agents. *Clin Infect Dis* 1997; **25:** 1430–40.

137 US Food and Drug Administration. FDA drug safety communication: FDA requires label changes to warn of risk for possibly permanent nerve damage from antibacterial fluoroquinolone drugs taken by mouth or by injection, 2013. http://www.fda.gov/Drugs/DrugSafety/ucm365050.htm (accessed May 18, 2015).

138 US Food and Drug Administration. FDA drug safety communication: azithromycin (zithromax or Zmax) and the risk of potentially fatal heart rhythms, 2013. http://www.fda.gov/Drugs/DrugSafety/ucm341822.htm (accessed May 18, 2015).

139 Berkelman RL. Consideration of prophylactic antibiotic therapy during an outbreak of Legionnaires' disease. *Clin Infect Dis* 2014; **58:** 144–45.

140 Sehulster L, Chinn RY, and the CDC, and the HICPAC. Guidelines for environmental infection control in health-care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). *MMWR Recomm Rep* 2003; **52:** 1–42.

141 Stout JE, Muder RR, Mietzner S, et al, and the Legionella Study Group. Role of environmental surveillance in determining the risk of hospital-acquired legionellosis: a national surveillance study with clinical correlations. *Infect Control Hosp Epidemiol* 2007; **28:** 818–24.

142 American Society of Heating, Refrigerating and Air-Conditioning Engineers. Guideline 12–2000: minimizing the risk of legionellosis associated with building water systems. Atlanta, GA; 2000. http://www.techstreet.com/products/232891 (accessed June 1, 2014).

143 Martin D. Air and water: hospitals should prepare now for ASHRAE's upcoming *Legionella* standard. *Health Facil Manage* 2012; **25:** 41–43.

144 Stout JE, Yu VL. Experiences of the first 16 hospitals using copper-silver ionization for *Legionella* control: implications for the evaluation of other disinfection modalities. *Infect Control Hosp Epidemiol* 2003; **24:** 563–68.

145 Garrison LE, Demirjian A, Lucas CE, et al. A large healthcare-associated legionnaires' disease outbreak in the setting of routine environmental testing for *Legionella*: the importance of disease surveillance. In: The 8th international conference on *Legionella*; Melbourne, Australia; Oct 29–Nov 1, 2013. Abstr 19.

146 Mandell GL, Bennett JE, Dolin R. Mandell, Douglas and Bennett's principles and practice of infectious diseases, 7th edn. Philadelphia, PA: Churchill Livingstone Elsevier, 2010.

147 Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW. Manual of clinical microbiology, 10th edn. Washington, DC: American Society of Microbiology Press, 2011.

148 Garcia LS. Clinical Microbiology Procedures Handbook, 3rd edn. Washington, DC: American Society for Microbiology Press, 2010.

149 Goldman L, Schafer AL. Goldman's Cecil medicine, 24th edn. Philadelphia, PA: Elsevier Saunders, 2010.

150 Marre R, Kwaik YA, Bartlett C, et al. *Legionella*, 1st edn. Washington, DC: American Society for Microbiology Press, 2002.

151 Heuner K, Swanson M, eds. *Legionella* molecular microbiology, 1st edn. Norfolk: Caister Academic Press, 2008.