

Epidemiology and clinical management of Legionnaires' disease



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Legionnaires' disease is an important cause of community-acquired and hospital-acquired pneumonia. Although uncommon, Legionnaires' disease continues to cause disease outbreaks of public health significance. The disease is caused by any species of the Gram-negative aerobic bacteria belonging to the genus *Legionella*; *Legionella pneumophila* serogroup 1 is the causative agent of most cases in Europe. In this Review we outline the global epidemiology of Legionnaires' disease, summarise its diagnosis and management, and identify research gaps and priorities. Early clinical diagnosis and prompt initiation of appropriate antibiotics for *Legionella* spp in all patients with community-acquired or hospital-acquired pneumonias is a crucial measure for management of the disease. Progress in typing and sequencing technologies might additionally contribute to understanding the distribution and natural history of Legionnaires' disease, and inform outbreak investigations. Control of Legionnaires' disease outbreaks relies on rapid ascertainment of descriptive epidemiological data, combined with microbiological information to identify the source and implement control measures. Further research is required to define the actual burden of disease, factors that influence susceptibility, key sources of infection, and differences in virulence between strains of *Legionella* species. Other requirements are improved, specific, sensitive, and rapid diagnostic tests to accurately inform management of Legionnaires' disease, and controlled clinical trials to ascertain the optimum antibiotics for treatment.

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Introduction

Legionnaires' disease is an important but relatively uncommon respiratory infection that can cause substantial morbidity and mortality. First recognised as a fatal cause of pneumonia more than three decades ago, only modest progress has been made in the investigation, clinical and incident management, and public health response to cases and outbreaks.¹ Legionnaires' disease, named after the 1976 American Legion convention in Philadelphia where it was first identified, is characterised by pneumonia that can be associated with generalised sepsis. Globally, most cases relate to *Legionella pneumophila*, although cases related to other legionella bacterium might not have been identified because appropriate assays are not available, or because an ascertainment bias exists; in many countries the primary diagnostic technique, urinary antigen testing, is poorly sensitive for strains that are non-*L pneumophila* serogroup 1 or other species, including *Legionella longbeachae*. In countries such as Australia, New Zealand, and Scotland where serology or PCR might be used as a primary diagnostic test, *L longbeachae* and other species have been identified as the cause of several infections.²

The substantial morbidity associated with Legionnaires' disease, its widespread occurrence, and recent major outbreaks emphasise the need for further research to support early diagnosis and improve clinical or outbreak management.^{3,4} This Review summarises the global epidemiology of Legionnaires' disease and its diagnosis and management, and identifies key knowledge gaps for the prioritisation of research.

Epidemiology

Surveillance schemes for Legionnaires' disease are in place in countries such as the USA, Canada, New Zealand, Australia, Japan, Singapore, and in Europe, where Legionnaires' is a notifiable disease and

coordinated European surveillance has been in place since 1995. However, data from other parts of the world are scarce (table 1). Legionnaires' disease is likely to be under-recognised in many countries because of a scarcity of common definitions, diagnostics, and surveillance systems. Reported data will probably be an underestimate and not directly comparable between countries. The global incidence of Legionnaires' disease is therefore difficult to quantify and care should be taken in interpretation of the surveillance data.

Globally, the age and sex distribution of cases are similar between countries. The disease is rare in children; most cases occur in older people (74–91% of patients ≥ 50 years) and cases are predominantly in men (1.4–4.3 male patients for every female patient).^{5–11} Within Europe, the age-standardised notification rate of Legionnaires' disease was 9.2 per million people in 2011, with wide variation among countries (range 0–21.4 per million).⁵ Apart from small year-on-year variations, these rates have not changed since 2005.⁵ The highest numbers of reported cases consistently occur in France, Italy, and Spain;^{5,12,13} however, the reported crude incidence of Legionnaires' disease in the USA increased from 3.9 to 11.5 per million between 2000 and 2009, with higher notification rates in the northeastern states than in other states.⁶

Legionnaires' disease shows a seasonal pattern, with peak activity in late summer to autumn (table 1). Several studies have linked this increase to warmer and wetter weather conditions, and higher relative humidity in these seasons.^{14–18} Evidence suggests increased survival of *L pneumophila* in aerosols at high relative humidity, although laboratory aerosol viability data might not represent true environmental survival.^{19,20} Australia and New Zealand observe an additional peak of cases of Legionnaires' disease during spring because of infection with *L longbeachae*, which is speculatively linked to compost use in gardening activities.^{8,11}

	Rate per 1 million people	Age distribution (% ≥50 years)	Male:female ratio	Case fatality rate (%)	Peak incidence months	Cases associated with travel abroad (%)
Europe ⁵	9.2 (0.0–21.4)†	77.0%	2.8:1	10.3%	July–Sept	13.0%
USA ⁶	10.8	74.0%	1.8:1	8.0%	June–Oct	≈4.6%
Canada ⁷	4.0	75.0%	1.57:1	NR	July–Oct	NR
New Zealand ⁸	14.0 (25.0)‡	Highest rate in 50–59 year age group	1.7:1	5.1%	Sept–Nov and March–May	6.5%
Japan ⁹	2.0–7.0§	90.8%	4.3:1	NR	June–Nov	NR
Singapore ¹⁰	6.5	76.1% (≥55 years)	1.4:1	2.2%	NR	27.3%
Australia ¹¹ (NNDSS 2012)	13.0	87.0–89.0% (≥45 years)	1.9:1	NR	Sept–Nov and March–May	NR

NNDSS-2012=National Notifiable Diseases Surveillance fortnightly summary notes-2012 (Australia). NR=not reported. *Legionnaires' disease is underestimated because of the substantial variation in case ascertainment, diagnostic approaches, and reporting practices; in particular, because of the diagnostic limitations in Singapore and Canada. Data are therefore not directly comparable between countries. †Range for all countries. ‡Laboratory-confirmed cases only. §Data are for the period 2005–09.

Table 1: Worldwide epidemiology of Legionnaires' disease*

A history of recent travel is associated with Legionnaires' disease, particularly overnight stays in hotel accommodation.²¹ Rooms being unoccupied for a long time and large numbers of water outlets with long pipe runs can result in water stagnation and *Legionella* spp growth unless adequate control measures are applied.²² Cruise ships can be sources of legionella for similar reasons and have been associated with outbreaks of Legionnaires' disease.²³

In view of the association with travel, specific surveillance systems for travel-related cases of Legionnaires' disease are in place to improve source identification and public health action.^{6,23} Notification rates and estimated risk to travellers varies by country; for example, 1.68 cases were noted per million nights spent in Greece in 2009, compared with 0.55 cases per million nights spent in the UK in the same year.²³

Transmission, natural history, and risk factors

Transmission of Legionnaires' disease is usually by inhalation of aerosols or aspiration of water containing *Legionella* spp; no evidence of person-to-person transmission exists. Legionnaires' disease due to *L longbeachae* is thought to have a different route of transmission, which is yet to be fully identified, but exposure to potting compost or soil, or gardening activities is regarded as a risk factor.^{24,25} Poor hand-washing practices after gardening, long-term smoking, and being near dripping hanging flower pots have also been associated with a greater risk.²⁶ Although most cases of Legionnaires' disease occur sporadically, clusters warranting investigation and point source outbreaks can occur, sometimes with substantial implications for public health.²⁷ Large outbreaks of Legionnaires' disease have been associated with contaminated cooling towers,^{27–29} hot and cold water systems, and whirlpool spas.^{30,31} Indeed, any source of aerosol generation has the potential to transmit *Legionella* spp, and a wide range of mechanisms and settings have been described that include contaminated hospital equipment, evaporative air conditioning units, cruise ships, and hotels. Cases of Legionnaires' disease

have also been linked to fountains, supermarket mist machines, and ice machines.^{32–34}

Although many people are exposed to *Legionella* spp, very few develop Legionnaires' disease (0.01–6.4%; USA, 1987 and 1993; Netherlands, 1999; Spain, 2002; German passengers on a cruise liner, 2003; Norway, 2005).^{31,35–39} Susceptibility to disease is associated with smoking, older age, chronic cardiovascular or respiratory disease, diabetes, alcohol misuse, cancer (especially profound monocytopenia as seen in hairy cell leukaemia), and immunosuppression.^{21,40–42} Infection with *Legionella* spp has emerged as a complication of anti-tumour-necrosis-factor (TNF)- α therapy with an increased risk compared with the general population, although less pronounced in patients receiving etanercept than in those receiving infliximab or adalimumab.⁴³

The incubation period of Legionnaires' is thought to be 2–10 days (median 6–7 days); however, longer and shorter incubation periods have been noted.^{28,31} For example, an incubation period of 19 days was noted in one outbreak, with 16% of cases having incubation periods of at least 10 days.³¹

A mortality rate of 8–12% is typical in most people but might be higher in people who are elderly, have pre-existing medical conditions, smoke, are nosocomial cases, or have a delay in diagnosis and treatment of their disease.⁴⁴ The average case-fatality rate is 10% in Europe (range 0–27% in countries reporting ≥ 30 cases) and 8% in the USA.^{5,6} The case-fatality rate in nosocomial cases is higher and ranges between 15% and 34%.^{5,6}

A mild, self-limiting, non-pneumonic, and non-fatal illness known as Pontiac fever has also been associated with exposure to aerosols containing *Legionella* spp. This disease has a short incubation period (between 5 and 66 h but usually 24–48 h) and duration (2–5 days), and is more common in younger people. Pontiac fever is usually identified only when cases occur as part of a cluster or outbreak,^{45–47} possibly because of the mild nature of symptoms, but also because of a lack of consensus on diagnostic criteria and case definition.

Diagnosis

L pneumophila was identified as the causative agent of Legionnaires' disease by detection of specific antibodies in patients of the 1976 Philadelphia outbreak.⁴⁸ For a period of several decades, the consensus was that serology, with standardised reagents and appropriate control sera, offered a reasonably sensitive and specific primary diagnostic method, which was, however, subject to controversies about the choice of antigen preparation method and whether whole or subclass-specific immunoglobulin concentrations should be measured.^{49,50}

In the UK, the most widely used assays between the early 1980s and mid 1990s were the indirect immunofluorescent antibody test (IFAT) and the rapid microagglutination test, with reagents for *L pneumophila* serogroup 1 prepared and distributed by the Public Health Laboratory Service. In the mid-1990s reagent production ceased, so laboratories in the UK that still wished to use serology had to use alternative sources. In 1999, an assessment of the available commercial kits showed that although *L pneumophila* serogroup 1 monovalent antigen might be suitable for routine use, polyvalent antigens for serogroup 1–6 were not specific enough and should not be used alone.⁵¹ This finding was reinforced by a review in 2008 (unpublished data, table 2) of data from 2109 positive sera from 1781 patients obtained in routine diagnostic laboratories and submitted to the national reference laboratory during a 54 month period. These sera had been tested with mainly enzyme immunoassays targeting *L pneumophila* serogroup 1–6, in the submitting laboratory. However, retesting of these sera in the National Legionella Reference Laboratory using the reference IFAT assay with monovalent antigens against *L pneumophila* serogroup 1, 6, and 8, in the presence of campylobacter blocking fluid (to eliminate this source of false-positive results⁵²) showed that few of the positive sera had any positive results in the monovalent reference assays, and only 62 (3.5%) had diagnostically significant titres.

Concern about the reliability of diagnoses with these commercial assays was not confined to the UK. In 1996, the US Centers for Disease Control and Prevention changed their case definition and excluded a single high antibody titre coupled with a clinician's diagnosis of pneumonia being used to diagnose a case.⁵³ Later, the European Centre for Disease Prevention and Control followed suit, although a significant rise in titre against *L pneumophila* serogroup 1 remains evidence of a confirmed case in both jurisdictions.^{54,55} Data estimate a positive predictive value of only about 50% with even the best commercial assay, especially in regions where *L pneumophila* serogroup 1 infection is less common.⁵⁶ Thus, most positive results obtained with these commercial kits are of no diagnostic value.

Although detection of *L pneumophila* antigen in the urine of patients as a diagnostic method for Legionnaires' disease was first described soon after the 1976 outbreak,⁵⁷ it was not widely accepted as a routine diagnostic method

	Serogroup 1 (%)	Non-serogroup 1 (%)	Total (%)
Titre 16	73 (4.1%)	34 (1.9%)	107 (6.0%)
Titre 32	32 (1.8%)	17 (1.0%)	49 (2.8%)
Titre 64	64 (3.6%)	8 (0.4%)	72 (4.0%)
Titre 128	16 (0.9%)	1 (0.1%)	17 (1.0%)
Titre 256	14 (0.8%)	1 (0.1%)	15 (0.8%)
Titre ≥512	14 (0.8%)	0 (0.0%)	14 (0.8%)
Titre 4-times rise	15 (0.8%)	1 (0.1%)	16 (0.9%)
Positive at any titre	228 (12.8%)	62 (3.5%)	290 (16.3%)
Significantly positive titre	59 (3.3%)	3 (0.2%)	62 (3.5%)

Sera obtained in routine diagnostic laboratories from November, 2004, to February, 2008 were retested with indirect immunofluorescent antibody assays with monovalent antigens against *Legionella pneumophila* serogroup 1–6 and 8 (unpublished data, TH).

Table 2: The number and percentage of 2109 sera submitted as positive for *Legionella pneumophila* in tests using polyvalent antigens, which were confirmed at various titres when retested using monovalent antigens

and incorporated in international case definitions until the mid-1990s.⁵⁸ Several reasonably reliable commercial kits are available for routine use,^{59,60} and urinary antigen detection now accounts for 70–80% of cases that are diagnosed in Europe and the USA.^{5,6} However, reliance on urinary antigen detection has limitations, the first and most substantial being the poor sensitivity of assays for legionellosis caused by non-*L pneumophila* serogroup 1 mAb3/1 positive strains. Sensitivity in routine use is, at best, 80–90% for the diagnosis of community-acquired Legionnaires' disease caused by *L pneumophila* serogroup 1 strains, but less than 50% for Legionnaires' disease caused by other *L pneumophila* strains.^{61,62} Preliminary studies of experimentally infected guinea pigs suggest that a 19 kDa peptidoglycan-associated lipoprotein common to all *Legionella* species is detectable in urine.⁶³ Although the applicability of this result to human beings is not yet known, development of an assay to detect this lipoprotein could be useful. Second, although urinary antigen kits are usually marketed as specific for *L pneumophila* serogroup 1, they can give positive results with other *L pneumophila* serogroups when antigen loading is high, so it cannot be assumed that two urinary-antigen-positive linked cases are caused by the same *L pneumophila* serogroup.^{61,62}

Culture and isolation of legionellae from clinical specimens is the diagnostic gold standard. Importantly, isolation of the infecting strain allows epidemiological typing to be done, which provides valuable data for the control and prevention of further cases. Sensitivity of culture varies widely among laboratories but, when clinical awareness is high, it is in the order of 50–80%.^{64–66} In a recent study, legionellae could be isolated from 66% of cases overall, and from 80% when a sample was taken within 2 days of admission to hospital.⁶⁶ The sensitivity of culture is likely to be higher in hospitalised patients with more severe disease than in those in the community

Panel: Diagnosis and treatment of Legionnaires' disease

Diagnosis

- Culture and isolation of legionellae from clinical specimens constitutes the gold standard for diagnosis
- Urine antigen testing is the most frequently used diagnostic test; however, assays have poor sensitivity for non-*Legionella pneumophila* serogroup 1 mAb3/1 positive strains
- Serology is not suitable for immediate clinical management
- Real-time PCR is now regarded as the molecular method of choice for detection of *Legionella* spp, theoretically offering specificity, sensitivity, and speed; this method should be used as soon as possible for early diagnosis
- Anyone presenting with symptoms of community-acquired or hospital-acquired pneumonia should be tested for Legionnaires' disease

Treatment

- The aim of therapy is eradication of any infection, and management of complications and any comorbidities
- Antibiotic therapy targeted for *Legionella* spp (ie, macrolides or fluoroquinolones) should be included in the initial management of severe community-acquired and hospital-acquired pneumonia until a specific microbiological diagnosis is made

because of increased microbial load in respiratory specimens. Use of selective agars and pre-treatments (heat or acid) to reliably isolate legionellae is not simple and, in view of the low prevalence of Legionnaires' disease, few laboratories now regard these methods as cost effective enough to use routinely. However, attempts to culture from urinary-antigen-positive patients and immunocompromised patients should be strongly encouraged because of the public health importance of obtaining isolates in urinary-antigen-positive patients and the greater likelihood of *L pneumophila* non-serogroup 1 infection in immunocompromised patients. Another major drawback to the culture method is that it takes 3–5 days to isolate the organism; the need to reduce this delay was key to the development of rapid molecular sequencing techniques.

The theoretical sensitivity of PCR testing (ie, detection of one copy of target sequence) quickly led to its widespread development. Real-time PCR is now regarded as the molecular method of choice for detection of *Legionella* spp, theoretically offering specificity, sensitivity, and rapidity of results (a few hours from collection of the sample). Most published data relate to *L pneumophila*-specific PCRs, which typically target the *mip* gene. These assays can detect infections of any *L pneumophila* serogroup, and have higher sensitivity than culture methods (around 15% increased yield compared with culture in the study by Mentasti and colleagues).⁶⁶ *Legionella* spp PCRs (usually targeting 16S rDNA) have been used in some studies and seem to offer greater sensitivity. However, in the CAPNETZ study,⁶⁷ although 10% of legionellosis was detected only with 16S rDNA *Legionella* spp PCR, only 60% of these could be confirmed by DNA sequencing,⁶⁷ suggesting that results obtained with such assays should be treated with caution until more specificity data are available.

The dry cough sometimes associated with Legionnaires' disease makes obtainment of respiratory samples difficult, and subsequently hampers outbreak investigations by severely limiting the number of cases with typing data. Early data from 5S rDNA PCR suggested that *L pneumophila* DNA could be detected in urine and sera at high sensitivity (>80%);^{68,69} however, results from studies with larger series of cases and different PCRs suggest that the sensitivity in serum is much lower (30–50%).^{66,70} In a series of 100 cases proven by urinary antigen, Mentasti and colleagues⁶⁶ did not obtain a positive result.

Clinical presentation and management

The high mortality and morbidity associated with untreated Legionnaires' disease means that the priorities for clinical management are: early diagnosis and prompt treatment with effective antibiotics; appropriate management of complications such as respiratory failure, renal failure, and CNS involvement; and the management of underlying comorbidities and risk factors (panel).^{71–77} Effective management is crucially dependent on clinicians considering the possibility of Legionnaires' disease in patients presenting with pneumonia or a multisystem illness with fever at all points of care.^{71,74}

Legionnaires' disease does not have specific, defining clinical features because it presents as a range of clinical manifestations and symptoms.^{71,75,76} These include: fever with organ-specific symptoms and signs, such as diarrhoea or confusion, or both; fever with multisystem disease including rhabdomyolysis with renal failure; community-acquired pneumonia; hospital-acquired pneumonia; pneumonia with extrapulmonary features; and severe fulminant disease. Previously, a weak clinical response to β -lactam antibiotics in patients with community-acquired pneumonia raised the possibility of Legionnaires' disease; however, this misdiagnosis should not now occur if guidelines that include the management of atypical pneumonias are adhered to.⁷²

A diagnosis of Legionnaires' disease should alert a physician to the possible existence of other cases related in place or time that might be crucial for identification of the potential source of infections.⁷⁷ For this reason, history taking should include a detailed enquiry of any potential exposure to aerosolised water droplets from a range of environmental settings (especially during the previous 10 days). A detailed history of the recent movements of the patient is recommended to support the epidemiological follow-up, trace any other patients, and identify the source of infection. Legionnaires' disease is a notifiable disease in many countries and cases should be reported immediately to the public health authorities.

Although Legionnaires' disease can occur in previously healthy individuals, it occurs more frequently in those with predisposing risk factors, such as smoking, chronic cardiovascular or respiratory disease, diabetes, alcohol misuse, and immunosuppression. Immunosuppressed

patients in particular might present with more severe clinical disease (often complicated by cavitation and pleural effusions), and frequently require intensive care, intravenous antibiotics, and a longer duration of therapy.^{78–81} Bilateral pulmonary involvement with high case fatality rates has been reported in patients with haematological malignancies.⁸²

Therapy for Legionnaires' disease is antibiotic treatment of the infection and management of complications and any comorbidities.⁷⁶ Recovery is most likely if the appropriate antibiotics are given early.^{72,75} In view of the fact that β -lactam antibiotics, usually used to treat bacterial community-acquired pneumonia, are ineffective for treatment of Legionnaires' disease, and that Legionnaires' does not have any defining clinical features, it is prudent to give empirical antibiotic therapy effective against *Legionella* spp in the initial management of all moderate-to-severe community-acquired and hospital-acquired pneumonias until a specific microbiological diagnosis is made.

The dose and route of administration of treatment, whether oral or intravenous, is guided by severity, underlying risk factors, consciousness level, and gastrointestinal disorders. Because *L pneumophila* is an intracellular pathogen residing within tissue and alveolar macrophages, successful treatment depends on use of antibiotics that achieve therapeutic intracellular concentrations within macrophages, such as the macrolides, fluoroquinolones, and cyclin families.^{74,83–87} Erythromycin had been the drug of choice for treatment of Legionnaires' disease until the 1990s but is now used less often because it is bacteriostatic and has side-effects, particularly when used intravenously.^{1,88} However, the newer macrolides such as azithromycin and tetracyclines have fewer side-effects. Fluoroquinolones are bactericidal and their in-vitro activity against *Legionella* spp in animal models is superior to that of erythromycin.^{89,90} Antibiotics recommended for the treatment of Legionnaires' disease are azithromycin or levofloxacin. These drugs are highly effective, have become the mainstay of antilegionella therapy in healthy and immunocompromised individuals, and are preferable to erythromycin because of fewer side-effects.^{91–93}

Combination therapy has been advocated to treat severe Legionnaires' disease, possibly because of the relative ineffectiveness of erythromycin monotherapy, but there is no evidence of superiority of dual antibiotic therapy versus monotherapy, and insufficient rationale to add a second drug even in severe cases of Legionnaires' disease.^{74,92}

Several specialist society guidelines have been published that cover the treatment of Legionnaires' disease within the recommendations for community-acquired pneumonia. The Infectious Diseases Society of America/American Thoracic Society (IDSA/ATS) guidelines were issued in 2007,⁷⁴ the British Thoracic Society (BTS) in 2009,⁷⁵ and the Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS) in 2012.⁹⁴

IDSA/ATS guidelines recommend that patients with Legionnaires' disease should receive a minimum of 5–14 days therapy, with a shorter course if azithromycin is used because of its long half-life. Treatment should not be stopped until patients have been afebrile for 48–72 h. Duration of antibiotic use should always be calibrated with clinical response and improvement in biomarkers, with an appropriate extension of up to 21 days (10 days for azithromycin) in immunocompromised patients. Complications (eg, extrapulmonary infection, such as meningitis or endocarditis) might warrant longer therapy.^{74,89,95}

Investigation of outbreaks and typing of *Legionella* spp

Investigation of Legionnaires' disease outbreaks is driven by the potential for a point-source to expose large numbers of the population to contaminated aerosols that might be dispersed over a wide area. Several high profile incidents of such exposures have occurred,^{27,29,96} and are a particular issue for cooling towers where previous outbreak investigations established evidence of infection 10–15 km from the source.^{38,97,98} However, many outbreaks show shorter distance dispersion,^{27,96} and environmental conditions and physical geography probably play a part in determining dispersal distances. Effective surveillance and notification systems are crucial to the early identification of outbreaks, which can evolve explosively and produce hundreds of cases within days.^{27,31,96} Table 3 summarises a selection of notable Legionnaires' disease outbreaks delineating the variety of sources and numbers of cases. Control of outbreaks of Legionnaires' disease relies on rapid ascertainment of descriptive epidemiological data, combined with microbiological information, to identify the source and implement control measures. The low case-fatality rates in several recent outbreaks have been attributed to rapid investigation and the implementation of control measures.^{3,96}

Detailed case histories with standardised questionnaires are necessary to build an epidemiological picture and identify links in time and location. If necessary, subsequent trawling questionnaires could also be used. On the basis of case histories, sources should be identified and risk assessments done to guide and prioritise investigations and environmental sampling. The microbiological aspect of any investigation is to seek evidence linking the source of the outbreak to the cases, by comparison of *Legionella* isolates in environmental samples with those from patients.

Isolates of *L pneumophila* serogroup 1 can be rapidly subgrouped with monoclonal antibody panels based on the international monoclonal antibody subgrouping panel.^{106,107} However, this method has poor discrimination, and divides *L pneumophila* serogroup 1 into only eight to ten phenons, so consequently, many other methods have been investigated. A DNA-sequence based typing method, which discriminates *L pneumophila* into more than

	Number of cases	Fatalities (%)	Confirmed or suspected source	Key features
Philadelphia, USA (1976) ¹	182	15.9%	No source confirmed; visiting the hotel lobby was a risk factor	An outbreak of, at the time, an unrecognised form of pneumonia at an American Legion convention. The outbreak led to the identification and characterisation of legionella and Legionnaires' disease
Los Angeles, USA (1977–82) ⁹⁹	>200	..	Potable water system	An ongoing outbreak at a hospital in Los Angeles. More than 200 cases occurred across a 5-year period, with a peak in March, 1980, when 26 cases were identified in 1 month. Legionella of the same serogroups as clinical isolates were found in samples from the sinks and showers in patient rooms. The number of cases dramatically and sustainably decreased after the water system was hyperchlorinated
Stafford, UK (1985) ¹⁰⁰	68	32.4%	Air conditioning plant	A large nosocomial outbreak
London, UK (1988) ⁷⁷	70	4.3%	Cooling towers	Outbreak due to <i>Legionella</i> species infections in cooling towers at BBC Broadcasting House, central London
Cruise ships, (1994) ¹⁰¹	50	..	Whirlpool spa on board ship	Cases occurred across nine cruises from April to June, 1994
Bovenkaspel, Netherlands (1999) ³¹	188	11.0%	Whirlpool spa on display	Outbreak due to a whirlpool on display at a busy flower show. Incubation periods that were longer than average were noted; 16% of cases had incubation periods of more than 10 days
Melbourne, Australia (2000) ²⁸	125	3.2%	Cooling tower	Outbreak among visitors and passers-by of an aquarium. Long incubation periods were noted, ranging from 1 to 16 days
Murcia, Spain (2001) ²⁷	449	1.1%	Cooling tower at hospital	Community outbreak with the largest number of cases confirmed by microbiology, and up to 800 possible cases
Barrow-in-Furness, UK (2002) ²⁹	179	3.9%	Cooling tower	Largest UK outbreak
Fredrikstad, Norway (2005) ³⁸	56	17.8%	Air scrubber	Evidence of long-distance spread of legionella, with infections reported 10 km from source
Christchurch, New Zealand (2005) ⁹⁸	19	15.8%	Cooling tower	Evidence of long-distance spread of legionella, with cases detected up to 11.6 km from the suspected source
Rapid City, USA (2005) ³²	18	5.6%	Decorative fountain	A small decorative fountain was implicated as the cause of an outbreak
Pamplona Spain (2006) ⁹⁶	146	0.0%	Cooling towers	Outbreak with an unusually low case fatality rate, because of early detection and prompt medical and public health action
Pas-de-Calais, France (2006) ⁹⁷	86	21.0%	Industrial cooling towers	Outbreak provided evidence of long-distance spread from a powerful industrial cooling tower, with a high fatality rate.
Miyazaki, Japan (2008) ¹⁰²	295	2.4%	Public bathhouse	Outbreak in a public bathhouse with a circulating system (total includes suspected cases)
Las Vegas, USA (2001–08) ¹⁰³	35	5.3%	Potable water system	An ongoing outbreak in a Las Vegas hotel; transmission was associated with duration of showering
Corfu, Greece (2011) ¹⁰⁴	15	6.7%	Multiple sources	A cluster of cases and small outbreaks very close in location and time; initially thought to be a single point-source outbreak
Edinburgh, UK (2012) ³	50	4.3%	No source confirmed, cooling tower cluster suspected	49 further suspected cases were noted over a wide geographical spread. Plume modelling was used to identify the source because environmental investigations were inconclusive
Stoke-on-trent, UK (2012) ⁴	21	9.5%	Whirlpool spa on display	Recent outbreak due to a whirlpool spa on display at a shop
Quebec, Canada (2012) ¹⁰⁵	181	7.7%	Cooling tower	Outbreak, linked to a cooling tower attached to a fast food restaurant

Table 3: Selection of notable worldwide outbreaks of Legionnaires' disease from 1976 to 2012

1700 types, has now become the international standard together with its associated online database.¹⁰⁸ A major advantage of this method is that epidemiological typing data can be obtained directly from clinical samples without the need to culture and isolate the organism.^{66,109} A combination of *L pneumophila* PCR and direct sequence-based typing enables highly discriminatory typing data to be obtained from clinical samples in less than 24 h.⁴

Epidemiological typing is extremely important in linking cases to a source; however, it can incorrectly link a source to a case if not accompanied by careful investigation. Epidemiological typing can also help to identify pseudo-outbreaks that are actually clusters of unrelated cases caused by different strains.^{104,110} In the absence of clinical samples, environmental samples are still useful to confirm or rule out the presence of *Legionella* spp. Combined with a risk assessment of the system, this information can help to establish the likelihood of that system as a source. Spatial analysis and mathematical

modelling techniques such as cluster analysis, infection window analysis, plume-modelling, and attack ratio analysis can be used to enhance the more traditional investigation techniques and help to direct investigations; however, these techniques still rely on good case data.^{31,98,111,112}

Analysis of international epidemiological typing data shows that most Legionnaires' disease is caused by a relatively small subset of all legionella strains recovered from the environment.^{113,114} Although some strains (eg, ST1) are widespread, others seem to be restricted to particular regions (eg, ST47 in northern Europe). This uneven distribution sometimes limits the methods' usefulness and has led to the development of additional typing methods such as insertion sequencing, variable element typing, and spoligotyping.^{115–117} However, these methods are not amenable to standardisation and so have not been widely applied.

Recently, whole-genome sequencing has been explored as a possible method for typing of *L pneumophila* and

proof of principle studies have shown that whole-genome sequencing has the potential to be the ultimate typing method,¹¹⁸ although currently it can be applied only where isolates have been obtained. Much work is still needed, however, to refine the data processing pipelines for whole-genome data before a truly standardised typing method can be established.

Research priorities

Despite the clinical consequences of Legionnaires' disease, little progress has been made in the past 30 years to appropriately define the burden of disease, the factors that affect susceptibility, key sources of infection, and differences in the virulence of strains. Additionally, understanding of the optimum methods for treatment and environmental control of this disease, and how to assess risk and investigate clusters or outbreaks is scarce. The following research priorities are based on an assessment of the published literature and the expert view of this group of authors.

Work is needed to better estimate the incidence of Legionnaires' disease, and to quantify the associated morbidity and mortality, and the economic burden, to appropriately prioritise resources to prevent and control outbreaks. Data from studies in Europe^{67,119} suggest that 2–5% of cases of community-acquired pneumonia are actually Legionnaires' disease, which is around ten times higher than reports received through even the best national surveillance systems.

Although some evidence is available for who gets infected and where, a further understanding of the population at risk would be useful. For example, will new biotherapies for managing systemic inflammatory diseases promote the occurrence of legionellosis, as seen with treatment with TNF- α blockers? At present, attack rates seem to be extremely low in recognised incidents. There is good evidence of genetic predisposition in animals (eg, mice) and emerging evidence of genetic risk factors in people.¹²⁰ Genetic, molecular, and epidemiological data are needed to explore whether the sex and age differences represent ascertainment bias, true susceptibility, or behavioural risks (such as smoking and occupational exposure). Research into better and more efficient public health systems to rapidly investigate clusters of cases is needed.

Geographical information systems analysis, typing, sequencing technologies, and mathematical modelling techniques are increasingly used to augment the traditional methods of investigation. Geographic information systems have proven useful to rapidly show the outbreak situation, and have been used to identify potential sources, direct further investigations, or corroborate findings.^{98,121} Mathematical models to predict the future course of outbreaks and direct public health actions might prove useful in the control of, and response to, outbreaks.¹¹⁴ Plume modelling has been used to implicate a hypothesised source in the absence of

microbiological data.³ All these techniques, including next-generation sequencing, represent promising new methods in the arsenal of investigators. Further refinement of these methods based on new knowledge, data, and experience would be beneficial.

Data for mortality rates are needed to establish 30 day and longer-term survival, either through a population study or through existing surveillance mechanisms linked to death registration data. The cohort recruited in a potential population study might provide information about long-term sequelae with appropriate follow-up, and allow the collection of data for quality of life and economic and societal costs, to improve estimates linked to specific incidents.¹²²

Further research to improve the diagnostic accuracy of tests is also needed. The CAPNETZ study⁶⁷ reports that about 10% of legionellosis cases are caused by non-*L pneumophila* strains. Urinary-antigen testing is estimated to be no more than 80% sensitive for *L pneumophila* serogroup 1 mAb3/1 positive strains, 40% for *L pneumophila* serogroup 1 mAb3/1 negative strains, and around 20% for non-serogroup 1 strains. Population level data for non-mAb3/1 positive *L pneumophila* serogroup 1, non-serogroup 1, and non-*L pneumophila* are essentially absent. Development and validation of improved molecular assays provides the best opportunity to meet the rapidity and diagnostic accuracy required to guide disease management.

Prospective randomised controlled clinical trials examining which class of antibiotics is superior, or whether mono or dual antibiotic therapy is preferable, are also scarce. Such trials will be challenging because of operational difficulties with collecting the required number of patients, and limitations of undertaking prospective clinical trials in patients with acute illness without a confirmed microbiological diagnosis.

Data suggest that some strains of *Legionella* spp are more likely to cause infection than others;^{113,114,123} a representative, well designed, and adequately powered study of the environmental distribution of strains is essential to find out whether this hypothesis is valid. Additionally, next generation sequencing could allow the exploration of genotypic factors in disease causation.

Good evidence from studies suggests that some strains survive in aerosols better than others and, at least for *L pneumophila* serogroup 1 strains, this finding correlates with the mAb3/1 positive and mAb3/1 negative strains (which relates to the degree of 8-O-acetylation of the lipopolysaccharide).^{124,125} Further environmental studies should investigate phenotypic factors such as optimum growth temperature and aerosol survival that might distinguish more infectious strains from others.

Very little progress has been made so far with research into the ecology of *L pneumophila*. The control and eventual eradication of Legionnaire's disease would depend on our ability to measure the risk in different environmental settings. Present guidance relates to the presence or absence of *Legionellae*—would it be better to

Search strategy and selection criteria

We searched PubMed and Google Scholar from Jan 1, 1976, to January 31, 2014, for all publications in English with the terms "legionella", "legionellosis", "Legionella pneumophila", "Legionella longbeachae", "diagnosis", "transmission", "etiology", "typing", and "epidemiology". We also searched relevant references within previous reviews that were identified with this strategy.

focus on just *L pneumophila*, or specific strains? What about non-serogroup 1 strains (eg, serogroup 2–14) and other *Legionellae*? Could we better assess Legionnaires' disease risk by monitoring protozoa in a system rather than *Legionellae*? Because environmental control measures rely on temperature control, further research into the risk of scalding,¹²⁶ which accounts for an ageing population would be useful. All sources of infection (eg, heavy rainfall and the role of potting compost beyond *L Longbeachae*) should be explored. Research into the amoeba and *Legionellae* in various systems, materials, and biofilms would inform where particular strains can be detected and identify the factors that affect the presence or absence and quantity of these strains. Determination of these factors might, in turn, allow approaches to be developed that eradicate *Legionellae* from water systems.

Countries with surveillance systems should institute environmental surveillance to obtain national legionella incidence data from water testing. These data could be supplemented with data from selected enhanced sampling with full characterisation of isolates.

The sporadic nature of Legionnaire's disease and the infrequent occurrence of outbreaks have led to a situation for which investment in research and clinical awareness of this disease is low. Physicians should be aware of, and include, Legionnaire's disease in the differential diagnosis of patients with pneumonia. Funding bodies to prioritise research into the diagnosis, treatment, and control gaps identified for Legionnaires' disease are urgently needed

Contributors

IA, TH, and NP suggested and agreed the outline for the Review. FPF, AZ, IA, TH, and NP wrote the first draft. All authors contributed to the writing of the manuscript.

Declaration of interests

We declare no competing interests.

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