REVIEW

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Pathogenicity of the family *Legionellaceae*

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Abstract

The *Legionellae* are Gram-negative bacteria able to survive and replicate in a wide range of protozoan hosts in natural environments, but they also occur in man-made aquatic systems, which are the major source of infection. After transmission to humans via aerosols, *Legionella* spp. can cause pneumonia (Legionnaires' disease) or influenza-like respiratory infections (Pontiac fever). In children, Legionnaires' disease is uncommon and is mainly diagnosed in children with immunosuppression. The clinical picture of *Legionella* pneumonia does not allow differentiation from pneumonia caused by others pathogens. The key to diagnosis is performing appropriate microbiological testing. The clinical presentation and the natural course of Legionnaires' disease in children are not clear due to an insufficient number of samples, but morbidity and mortality caused by this infection are extremely high. The mortality rate for legionellosis depends on the promptness of an appropriate antibiotic therapy. Fluoroquinolones are the most efficacious drugs against *Legionella*. A combination of these drugs with macrolides seems to be promising in the treatment of immunosuppressed patients and individuals with severe legionellosis. Although all *Legionella* species are considered potentially pathogenic for humans, *Legionella pneumophila* is the etiological agent responsible for most reported cases of community-acquired and nosocomial legionellosis.

Key words: Legionnaires' disease, Pontiac fever.

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THE *LEGIONELLAE*: THEIR ENVIRONMENT AND IMPORTANCE

The search for the cause of the large outbreak of severe pneumonia that affected attendees of a convention of war veterans in Philadelphia in 1976 led to the discovery of a novel bacterium. The fastidious Gram-negative bacillus was named *Legionella pneumophila* (*Legionella* to honor the stricken American legionnaires and *pneumophila* meaning "lung-loving") (Fang et al. 1989). Three years later the first diagnosed case of an infection by this bacterium was reported in a two-and-a-half-year-old boy with acute lymphocytic leukemia (Ryan et al. 1979). Fifty-two species and 72 serogroups of bacteria which constitute the *Legionellaceae* family have been isolated so far in both natural and clinical conditions (Euzeby 2008). The *Legionellaceae* family belongs to the γ-subdivision of the *Proteobacteria*, in which the *Legionellales* order has been created. The *Legionellales* order consists of two families: *Legionellaceae* and *Coxiellaceae*. On the basis of differentiating phenotype features and DNA homology, three genera have been isolated within the *Legionellaceae* family: *Legionella*, *Fluoribacter*, and *Tatlockia*. In the *Coxiellaceae* family, two genera have been described: *Coxiella* and *Ricketsia*. The common characteristic of these bacteria is that they are intracellular parasites infecting protozoa, invertebrates, animals, and man (Brenner et al. 2005).

Legionellae are capable of existing in fresh waters and soil and are ubiquitous in natural environments. Their proliferation is favored by elevated temperature, the presence of certain organic and inorganic components (e.g. iron) in water, and the presence of other organisms. Water temperatures between 20 and 45°C stimulate the growth of *Legionellae*. No growth is observed below 20°C and survival is inhibited above 60°C. Their widespread survival in nature can be attributed to their relationships with algae, bacteria, and, particularly, protozoa. *Legionellae* are phagocytized by protozoans and subsequently reproduce within these organisms. These relationships provide nutrients and protec-

tion in harsh environmental conditions and also make the bacteria more virulent and antibiotic resistant. The adaptation of *Legionella* to intracellular niches within aquatic protozoa seems to have generated a pool of virulence traits during its evolution which allow *Legionella* to infect human cells as well.

The bacteria are also found in biofilms in man-made hot water systems such as taps, showerheads, air conditioning systems, cooling towers, spas, ice machines, vegetable mixers, fountains, dental devices, nebulizers, and hot water system pipes. The above-mentioned aerosol-generating systems aid in the transmission of *Legionella* from water to air. Human inhalation of contaminated aerosols leads to *Legionella* infections and the bacteria can cause two kinds of diseases. The severe form is the potentially fatal atypical pneumonia which is referred to as Legionnaires' disease. A milder flu-like form is known as Pontiac fever (Fields et al. 2002). Person-to-person transmission of Legionnaires' disease has not been reported in the literature.

Legionella is recognized around the world as an important cause of community-acquired pneumonia (CAP) and hospital-acquired pneumonia, occurring both sporadically and in outbreaks, and as the cause of Pontiac fever outbreaks. Infection with *Legionella* ranks among the three most common causes of severe pneumonia in the community setting, and the bacterium is isolated in 1–40% of cases of hospital-acquired pneumonia (Sabria and Campins 2003). Its severity and mortality rate are similar to those of pneumonia caused by *Streptococcus pneumoniae*. *Legionella* has also been found to be a common cause of CAP in patients requiring intensive care unit (ICU) admission and ventilatory support (Lück et al. 1994; Vergis et al. 2000). In the USA the sporadic form of the disease causes around 10,000 cases of pneumonia per year in adults requiring hospitalization, and perhaps the same number of cases of pneumonia in adults not requiring hospitalization (Breiman and Butler 1998; Marston et al. 1997). In a review of 41 prospective studies conducted in Europe which sought to identify the etiology of CAP, *L. pneumophila* was identified as the causative pathogen in 1.9% of outpatients, 4.9% of hospitalized patients, and 7.9% of ICU patients (Woodhead 2002). On the other hand, studies conducted recently in Germany showed that *Legionella* pneumonia occurred at identical frequencies in outpatients and inpatients (3.7% vs. 3.8%) (Von Baum et al. 2008). The incidence of legionellosis in Europe amounted to 11.2 cases per 1 million inhabitants in 2006 (Ricketts and Joseph 2007). In Poland, 97 cases of legionellosis have been reported this year (www.pzh.gov.pl). However, the number of actual cases is probably underestimated due to the difficulty in bacterial isolation from clinical samples. Moreover, many species and serogroups cannot be identified accurately by contemporary commercially available microbiological tests (Roig and Rello 2003). The *Legionella* urinary antigen test, which is widely used as a diagnostic screening test for *Legionella* infection, does not reliably detect infection due to *Legionella* species other than that caused by *L. pneumophila* serotype 1. Different assays have been evaluated, with sensitivities for the detection of other serogroups ranging from 14 to 69% (Den Boer and Yzerman 2004). It is assumed that only 2–10% of the estimated cases of Legionnaires' disease are reported (Sabria and Campins 2003). This may be particularly true for the pediatric population, because pediatricians tend to associate legionellosis with adult patients and routine laboratory studies do not normally detect *Legionella*.

The number of reported cases of legionellosis is significantly lower in children than in adults. Wide-scale studies carried out in the USA from 1990 through 2005 showed that only 1.7% of all cases of Legionnaires' disease were reported in pediatric age groups; 0.93% of the cases were reported in children aged ≤14 years. Most pediatric cases were reported in children 15–19 years old (44.3%), followed by infants aged <1 year (18.1%) (Neil and Berkelman 2008).

SPECIES AND SEROGROUPS ASSOCIATED WITH THE DISEASE

Legionella pneumophila is the most frequent species isolated from adult and pediatric patients with either community- or hospital-acquired legionellosis. There are 16 serogroups of *L. pneumophila*, two each in *L. bozemanae*, *L. longbeachae*, *L. feeleii*, *L. hackeliae*, *L. sainthelensi*, *L. spiritensis*, *L. erythra*, and *L. quinlivanii* and a single serogroup in each of the remaining species. In Europe, approximately 70% of isolates from Legionnaires' disease patients are typed as *Legionella pneumophila* serogroup 1 (Joseph 2004). Non-serogroup 1 *L. pneumophila* cause only 7% of legionellosis cases (particularly serogroups 4 and 6) (Yu et al. 2002). Nevertheless, cases of severe infections caused by other serogroups have been reported recently (Faris et al. 2005; Furugen et al. 2008; Guillouzouic et al. 2008; Lück et al. 2008). In contrast to the situation in Europe and the USA, *L. longbeachae* accounted for 30.4% of cases of community-acquired legionellosis in Australia and New Zealand (O'Connor et al. 2007). *L. longbeachae* is also frequently associated with severe pneumonia in rural Thailand due to exposure to contaminated potting soil mixes (Phares et al. 2007).

L. pneumophila serogroup 1 can be further divided into multiple subtypes using a variety of serologic, phenotypic, and genetic methods (Diederen 2008). Tests using monoclonal subtyping show that the strains of *L. pneumophila* serogroup 1 most commonly associated with the disease in humans share a common epitope (Dournon et al. 1988). Depending on the typing scheme used, these strains may be referred to as Pontiac (Watkins et al. 1985), monoclonal antibody (mAb) 2 reactive (Harrison et al. 2007; Joly et al. 1986), or mAb 3/1 positive (Borchardt et al. 2008; Helbig et al. 2002). In a European-wide study of *L. pneumophila* serogroup

1, approximately 66.8% of cases were mAb3/1 positive and 11.7% of the overall number of isolated strains belonged to the mAb-3/1-negative serogroup 1 subgroup (Helbig et al. 2002). In other studies, 79.6% of clinical isolates and only 12.8% of environmental isolates were subgrouped as mAb2 positive (Harrison et al. 2007). An immunochemical analysis of the epitope suggested that the increased hydrophobicity of these strains leads their enhanced survival in aerosols (Dennis and Lee 1988).

In recent years, genetic methods for typing *Legionellae* have become epidemiologically important. These include ribotyping, amplified fragment length polymorphism analysis, restriction fragment length polymorphism (RFLP) analysis, restriction endonuclease analysis, multilocus sequence typing, arbitrarily primed PCR, and pulsed-field gel electrophoresis (Drenning et al. 2001; Fry et al. 2002; Harrison et al. 2006; Saunders et al. 1990; Scaturro et al. 2005). The aim of studies using various genetic techniques is to determine the genotypic markers of virulence which would enable long-term monitoring of *Legionella* in the environment, thus preventing further cases. For example, an almost 20-year-long study conducted in the UK by Harrison et al. using RFLP analysis showed clear differences between the populations of clinical and environmental isolates. The clinical isolates showed less diversity than environmental ones, suggesting that the former were a subset of the latter. These data suggest that some strains of *L. pneumophila* are more likely to cause disease than would be expected from their relative frequency in the environment (Harrison et al. 2007).

In addition to *L. pneumophila*, 20 *Legionella* species have been documented as human pathogens on the basis of their isolation from clinical material. The most commonly isolated non-pneumophila *Legionella* spp. are *L. longbeachae* (3.9%), *L. bozemanae* (2.4%), *L. micdadei* (0.6%), *L. dumoffii* (0.6%), *L. felleii* (0.4%) *L. wadsworthii* (0.2%), and *L. anisa* (0.2%) (Yu et al. 2002). *L. dumoffii*, *L. micdadei*, *L. gormanii*, and *L. bozemanae* were isolated from pediatric cases and *L. waltersii* was identified as a cause of severe pneumonia for the first time in a 5-year-old girl (Greenberg et al. 2006; König et al. 2005). The majority of confirmed infections involving non-pneumophila *Legionella* species occurred in immunosuppressed patients (i.e. patients treated with a high-dose corticosteroid therapy or those with an organ transplant or a malignancy) and in splenectomized patients (Greenberg et al. 2006; Kümpers et al. 2008; Muder and Yu 2002).

Apart from the recognized *Legionella* species, a number of *Legionella*-related bacteria, designated as *Legionella*-like amoebal pathogens (LLAPs), have been described (Adeleke et al. 1996; Rowbotham 1993). The term LLAP refers to a subset of bacteria that grow in certain host species of amoeba and appear to be closely related to *Legionella* on the basis of 16S rRNA gene sequencing. Based on quantitative DNA hybridisation, LLAP-1 (*L. drozanskii*), LLAP-6 (*L. rowbothamii*), LLAP-10 (*L. fallonii*), and LLAP-12 (*L. drancourtii*) have been defined as four new *Legionella* spp. and LLAP-7 (fluorescent and non-fluorescent strains), LLAP-9, and LLAP-3 have been identified as members of the species *L. lytica* (Adeleke et al. 2001; La Scola et al. 2004). LLAP-3 was first isolated from the sputum of a patient with pneumonia after the clinical specimen was incubated with *Acanthamoeba polyphaga* (Fry et al. 1991). Serologic surveys of patients with pneumonia have suggested that LLAPs may be occasional human pathogens, both in hospitals and the community (Adeleke et al. 1996; McNally et al. 2000; Rowbotham 1993). *Legionella*-related endosymbionts (known as X-bacteria and renamed Candidatus *L. jeonii*) have been classified as belonging to the *Legionellaceae* family. The bacteria established a very specific mutual relationship with *Amoeba proteus* (Jeon and Jeon 2004; Park et al. 2004).

HUMAN DISEASES

Legionnaires' disease

Legionnaires' disease and Pontiac fever, both known as legionellosis, have been documented in children and adults with similar clinical effects. Legionnaires' disease begins after an incubation period of 2–10 days, although the incubation may extend over more than 10 days (Den Boer et al. 2002; Lettinga et al. 2002b). Early symptoms, often nonspecific, include a mild cold, low fever, malaise, anorexia, and muscle aches. About half of the patients develop puss-forming sputum and about one third develop blood-streaked sputum or cough up blood. Later symptoms are high fever, bronchiolitis, alveolitis, and lung damage with infiltrated regions (Bartram et al. 2007).

It is very difficult to clinically distinguish patients with Legionnaires' disease from patients with pneumococcal pneumonia or *Mycoplasma pneumonia* and *Chlamydophila pneumonia*. Several studies have shown that CAPs caused by *Legionella* and pneumocci have nearly identical clinical and radiological findings and that non-specific laboratory test results cannot reliably differentiate between the two diseases (Diederen 2008; Roig et al. 1991; Granados et al. 1989). Cunha published a weighted point evaluation scale, the Winthrop University Hospital (WUH) criteria, to identify Legionnaires' disease based on clinical criteria. In order to evaluate the utility of the WUH criteria, Gupta et al. performed a comparative study on groups of hospitalized patients known to have either Legionnaires' disease or pneumococcal pneumonia (Gupta et al. 2001). The WUH criteria correctly identified 29 of 37 patients with Legionnaires' disease (sensitivity: 78%, 95% confidence interval [CI]: 61–90%), while successfully excluding Legionnaires' disease in 20 of 31 patients with bacteremic pneumococcal pneumonia (specificity: 65%, 95% CI: 45–80%). The positive and negative predictive

values were estimated at 42% (95% CI: 25–61%) and 90% (95% CI: 74–97%), respectively. Nevertheless, there is some suggestion that a combination of various factors points to Legionnaires' disease rather than to other pneumonic diseases, but now studies have shown this unequivocally. However, in some circumstances clinicians can use selected clinical and nonspecific laboratory features to establish a suspected etiological diagnosis of Legionnaires' disease, although in the majority of cases this is not possible (Sopena et al. 1998). Gastrointestinal manifestations include watery diarrhea, nausea, vomiting, and acute generalized or localized abdominal pain, which are more consistent with Legionnaires' disease than with other pneumonic diseases (Cunha 2008a; Cunha 2006a; Garcia-Vidal and Carratala 2006). Hepatosplenomegaly has been observed in children and diarrhea has been reported in only 13% of pediatric patients (Greenberg 2005). Although diarrhea is also common for *Mycoplasma pneumoniae,* it does not occur in infections caused by *Streptococcus pneumonia*. In a study by Sopena et al. it was concluded that only diarrhea and elevated creatine phosphokinase (CK) levels are significantly discriminatory for CAP by *Legionella*, but their sensitivity was found to be very low (Sopena et al. 1998). *Legionella* is regularly accompanied by a pulse temperature deficit (Faget's sign), i.e. relative bradycardia, thus allowing to differentiate *Legionella* from other etiological agents causing CAP as well as *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* (Cunha 2008a). Relative bradycardia occurs with any *Legionella* species (Cunha 2008b).

Patients with Legionnaires' disease suffer from disorders related to the nervous system, the most common of which is a change in mental status. Other manifestations include headache, delirium, confusion, depression, hallucinations, gait disturbances, and lethargy to encephalopathy (Diederen 2008). Neurologic examination of children occasionally showed the presence of ataxia, tremor, hyperactive reflexes, encephalopathy, and the Guillain-Barre syndrome (Akyildiz et al. 2008; Esposito and Gantz 1986).

Hepatic involvement is common manifested by mildly increased serum transaminases in *Legionella* CAP. Mild elevations of serum transaminases also occur with Q fever and psittacosis, but they are not observed in infections with *Mycoplasma pneumoniae* or *Chlamydophila pneumoniae* (Cunha 2005a; Cunha 2005b). Other abnormalities include hyponatremia, hypophosphatemia, hematuria, CK elevation, serum creatinine and ferritin elevation, and proteonuria (Cunha 2006a, Cunha 2008c, Garcia-Vidal and Carratala 2006). Hyponatremia is a frequent finding in adult patients, but was seldom reported in pediatric cases (Greenberg et al. 2006). In contrast to hyponatremia, which may occur with a variety of pneumonias and noninfectious chest disorders, hypophosphatemia in CAP is associated only with Legionnaires' disease (Cunha 2006b). Hypophosphatemia occurs early and may be absent after 24 h of hospital admission, which is why this abnormality is easily overlooked. Microscopic hematuria is relatively more important than mild serum creatinine elevations in patients with *Legionella* (Cunha 2006a). Other nonspecific laboratory tests that are diagnostically useful in Legionnaires' disease are highly elevated levels of C-reactive protein (>35 mg/dl) and serum ferritin (2×n, n=220 ng/ml). A high serum procalcitonin level (0.5 μg/l) seems to be another marker of Legionnaires' disease (Haeuptle et al. 2009; Masia et al. 2007). Also, the diagnosis becomes more likely if an acute consolidating pneumonia fails to respond to several days of β-lactam antimicrobial therapy or if the pneumonia is severe enough to require ICU hospitalization (Fields et. al. 2002). The key to the diagnosis of Legionnaires' disease is to perform the appropriate microbiologic tests (Fields et al. 2002; Diederen 2008).

The symptoms and signs of the disease in children are non-specific; fever, cough, tachypnea, hypoxia, and changes in the lung are the most commonly encountered. Chest pain and hemoptysis are reported infrequently (Greenberg et al. 2006). In most cases of pediatric Legionnaires' disease, infection begins with febrile bronchiolitis and extra-respiratory symptoms appear to be more frequent than in adults (La Scola and Maltezou 2004). Neonatal legionellosis can present either as pneumonia or as nonspecific sepsis (Levy and Rubin 1998).

Clinical studies indicate that legionellosis in children resembles that in adults; nevertheless, Holmberg et al. cautioned that "the full spectrum of manifestations in children is unknown" (Holmberg et al. 1993). Several investigators have concluded that *Legionella* infection is common in school age children, and sero-epidemiological studies demonstrate that children are frequently exposed to *Legionella*. However, infection is usually asymptomatic or mildly symptomatic or self-limiting in immunocompetent children (Maltezou et al. 2004).

Radiographic changes

The radiographic pattern of Legionnaires' disease is not specific and there are no particular chest X-ray changes that are pathognomonic for *Legionella* CAP. However, certain abnormalities on the chest radiograph may indicate the presence of a *Legionella* infection. A chest radiograph characteristic of *Legionella* pneumonia demonstrates rapidly progressive asymmetrical infiltrates which are accompanied by pleural effusions (Cunha 2008a; Cunha 2008b). Pleural effusion has been a common finding among children. Pulmonary infiltrates on chest radiographs have been documented in 97% of children with Legionnaires' disease (Greenberg 2005). Another chest radiograph clue to Legionnaires' disease are worsening pulmonary infiltrates appearing in spite of an appropriate antimicrobial therapy (Cunha 2006a; Sugihara et al. 2007). In chest computed tomography scans, the most frequent appearances of *Legionella* pneumonia are multilobar or multisegmental

consolidations and ground-glass opacity (GGO) (Kim et al. 2007). Sharply demarcated peribronchovascular foci of consolidation intermingled with GGO also seem to be one of the most common abnormalities of *Legionella* pneumonia (Sakai et al. 2007). Cavitary lung disease is relatively more common in immunocompromised patients, including some groups of solid-organ transplant patients and neonates (Di Stefano et al. 2007; Quagliano and Das Narla 1993). Nevertheless, Famiglietti et al. reported two cases of legionellosis with cavitation in children who had no known underlying disease or immunodeficiency (Famiglietti et al. 1997).

Pontiac fever

Pontiac fever is an acute self-limiting non-fatal illness without pneumonia with a short incubation period and short duration. There is no agreed-upon definition of Pontiac fever nor has the exact pathogenesis of this disease been recognized. The detection of Pontiac fever may be a marker of an environment contaminated with *Legionella* and a risk of pneumonia. Recent studies conducted by Burnsed et al. which showed a high frequency (36%) of urine antigen-positive tests in the Oklahoma City Pontiac fever outbreak demonstrated that the epidemic was due in part to inhalation of live or dead *L. pneumophila* serogroup 1 and not just to inhalation of non-*Legionella* bacterial endotoxin (Burnsed et al. 2007; Edelstein 2007). The diagnosis of Pontiac fever is made on the basis of epidemiological, clinical, and environmental microbiology findings (Burnsed et al. 2007; Edelstein 2007; Tossa et al. 2006). Clinically, the symptoms of Pontiac fever mimic influenza, with fever, chills, headache, myalgia, malaise, nausea, nonproductive cough, abdominal pain, arthralgia, and a sore throat. Other symptoms, such as dyspnea, thoracic pains, vomiting, diarrhea, fatigue, and dizziness, have also been described (Tossa et al. 2006). Lüttichau et al. compared the clinical effects of Pontiac fever in children and adults (Lüttichau et al. 1997). Although the children had a shorter incubation period and longer duration of the illness than the adults, these differences were not significant. Arthralgia, myalgia, dizziness, low back pain, and chest pain were significantly more common in the adults, whereas ear pain and rash were significantly more common in the children. The children and adults exhibited no significant differences in the frequency of fever, headache, cough, abdominal pain, poor concentration, nausea, and a dry or sore throat or vomiting. Sequelae, consisting mainly of chest pain and poor concentration, were more common among the adults. The chest radiographs of both the adults and the children were normal (Lüttichau et al. 1997).

The epidemic of Pontiac fever among workers performing high-pressure cleaning at a sugar-beet processing plant reported by Castor gives additional insights into this disease. Thirteen of the fifteen workers affected in this outbreak had bronchoconstriction with respiratory compromise necessitating a nebulizer therapy. Oxygen was administered to 12 of them and all received antibiotic and corticosteroid therapy. The indicated respiratory findings seen in this outbreak are not typical of Pontiac fever (Castor et al. 2005).

EXTRAPULMONARY INFECTION

Extrapulmonary infection occurs rarely, either as disseminated infection in patients with pneumonia or, very rarely, as isolated primary infection, but its clinical manifestations can be severe. Infections in which *Legionella* species have been implicated include pancreatitis, peritonitis, cellulitis, sinusitis, perirectal abscess, and myositis (Eitrem et al. 1987; Stout and Yu 1997). The most commonly affected site is the heart (e.g. pericarditis, endocarditis, aortic graft involvement) and the kidney (e.g. pyelonephritis, glomerulonephritis) (Haluk et al. 2006; Pai et al. 1996; Shimura et al. 2008; Stout and Yu 1997). Reactive arthritis and osteomyelitis are uncommon manifestations, while myalgia and arthralgia during the acute phase of the infection are very common (Andereya et al. 2004; McClelland et al. 2004). *Legionella* rarely spread into the nervous system. More commonly, the disease leads to neurological conditions such as encephalomyelitis, brain abscess, cerebellum involvement, and peripheral neuropathy (Shelburne et al. 2004). In one case, cerebellar and frontal lobe hypoperfusion was reported in a patient with central nervous system Legionnaires' disease (Imai et al. 2008). *Legionella* meningoencephalitis may mimic the symptoms of herpes simplex encephalitis (Karim et al. 2002).

Long-term effects, up to 17 months after the diagnosis, may persist in an acute infection. Following an outbreak of Legionnaires' disease at a flower show in the Netherlands, the incidence of fatigue among the patients was 75% and the incidences of neurological and neuromuscular symptoms were 66% and 63%, respectively (Lettinga et al. 2002a). Extrapulmonary infection occurs also in children. For example, *L. micdadei* was isolated from the neck mass of a previously healthy 9-year-old girl (Qin et al. 2002).

RISK FACTORS

Environmental factors that increase exposure to the etiological agents are essential for the disease's transmission. Any water sources contaminated with *Legionella* spp., such as air conditioning, cooling towers, whirlpool spas, and water delivery systems, which function as amplifiers or disseminators of these bacteria, pose a risk of acquiring the disease. Certain demographic factors are associated with an increased susceptibility to Legionnaires' disease following exposure. Subpopulations at increased risk include extreme ages, i.e. neonates, due to their underdeveloped immune systems, and the elderly. Older infants and children share risk factors for community-acquired and nosocomial

legionellosis with those of adult patients. No additional risk factors specific to children have been reported.

A very important factor for the establishment of an infection with *Legionella* is the immune status of the host. The bacteria are normally cleared by an immunocompetent host. However, if the immune status is impaired, as is the case with individuals with diabetes, chronic lung diseases, or chronic severe renal failure, there is a high risk of the development of the disease. Other risk factors for community-acquired Legionnaires' disease include hematologic malignancies, lung cancer, male gender, alcohol consumption (Marston et al. 1994), administration of glucocorticosteroids, and some forms of immunosuppression (Lepine et al. 1998). Paradoxically, patients with AIDS appear not to be at an increased risk for Legionnaires' disease (Gutierrez Rodero et al. 1995). However, when they do develop a *Legionella* infection, these patients take longer to become afebrile, have more respiratory symptoms, and a higher rate of respiratory failure as well as mortality compared with patients with *Legionella* but without an HIV infection (Pedro-Botet et al. 2003; Sandkovsky et al. 2008). Cases of Legionnaires' disease among HIV-positive children have been reported. Nevertheless, the incidence of pneumonia caused by *Legionella* is extremely low in this population (Chaisson 1998; Stout and Yu 1997).

Patients with iron overload as well as smokers, whose lungs also contain elevated iron, are at an increased risk for Legionnaires' disease (Cianciotto 2007; Paradkar et al. 2008). Pregnant women are not at an increased risk of legionellosis, but certain cases have been reported during pregnancy (Eisenberg et al. 1997; Gaillac et al. 2006; Vimercati et al. 2000). These reports indicate that Legionnaires' disease during pregnancy may result in preterm delivery, and effects in the newborn may be secondary to preterm delivery. Tewari et al., however, observed that a neonate of a mother with Legionnaire's disease had a bowel perforation that may have been associated with an intrauterine infection (Tewari et al. 1997). Moreover, contaminated birthing pools have been the source of Legionnaires' disease in newborns after a water birth in a home bathtub and in a hospital birthing pool (Franzin et al. 2001; Franzin et al. 2004; Nagai et al. 2000).

The same risk factors which were mentioned for community-acquired *Legionella* infections seem to apply to nosocomial acquisition (Joseph et al. 1994). Some additional risk factors include recent surgery, intubation and mechanical ventilation, aspiration, as well as the use of respiratory therapy equipment and other medical devices. One of the highest incidence rates of nosocomial Legionnaires' disease is in the population of surgical head and neck cancer patients (Johnson et al. 1985). This group of people has a propensity for aspiration as a result of their oral surgery, which requires general anesthesia (Johnson et al. 1985). Nasogastric tubes have been linked to nosocomial legionellosis in several studies, with microaspiration of contaminated water as the presumed mode of entry (Venezia et al. 1994). A novel case of nosocomial *Legionella* related to transesophageal echocardiography probes contaminated by *Legionella* has been reported (Levy et al. 2003). As with adults, pediatric patients are at risk of contracting Legionnaires' disease in a hospital setting due to the use of aerosol generators, such as nebulizers and humidifiers. Neonates may be infected by the aerosol released from a humidifier in the incubator (Franzin et al. 2004).

Corticosteroids and disorders associated with immunosuppression (e.g. cancer therapy or organ transplant) are independent risk factors in adults and children (Poupard et al. 2007; Watson et al. 2004). In a study by Jacobson et al., the majority of cancer patients had an underlying hematological malignancy (82%) or another active malignancy (80%) and 37% were bone marrow transplant recipients (Jacobson et al. 2008). Most infections reported in children have been cases of patients with leukemia and isolated cases of astrocytoma and lymphoma (Trübel et al. 2002).

Transplant recipients carry the highest risk of nosocomial Legionnaires' disease (Singh et al. 2002). Heart and liver transplant patients have been shown to have a high incidence of Legionnaires' disease (Del Pozo 2008; Guyot et al. 2007; Mathys et al. 1999). Legionellosis has been reported in 2–17% of heart transplant recipients (Horbach and Fehrenbach 1990; Redd et al. 1988). In certain infections in liver transplant recipients, *Legionella* spp. are among the most common pathogens, perhaps connected with simultaneous splenectomy for associated hypersplenism (Singh et al. 1996). The lowest incidence concerns bone marrow transplants (Chown and Yu 1998). In children, cases of infection have been noted after kidney, liver, heart, bone marrow, and stem cell transplants (Campins et al. 2000; Gonzales and Martin 2007; Miller et al. 2007; Takunaga et al. 1992).

The association between corticosteroid use and the risk of infection with *Legionella* is also apparent in the large population of children with asthma. However, part of these cases may result from the use of respiratory equipment, including medical nebulizers. Other risk factors, particularly in children, include congenital heart disease, congenital immunodeficiency, respiratory diseases such as bronchopulmonary dysplasia, pneumothorax, follicular bronchiolitis, hyaline membrane disease, and tracheobronchomalacia (Greenberg et al. 2006). Treatment with glucocorticoids, tumor necrosis factor antagonists, or cytotoxic agents can also predispose individuals to Legionnaires' disease (Wondergem et al. 2004).

Apart from the already mentioned risk factors, severe *Legionella* infections have occurred among previously healthy people, including infants and young people without an underlying disease and those without other known risk factors (Falguera et al. 2001). In several studies of the etiology of community-acquired pneumonia, *Legionella* spp. have been identified as the causative agent in 1–5% of previously healthy children

(Orenstein et al. 1981). Pneumonia caused by *L. pneumophila* or *L. micdadei*, confirmed by PCR or using antibody detection methods, has been reported in immunocompetent children (Casaulta et al. 1999; Mrozińska 2005; Qin et al. 2002).

Comparative studies of the risk factors, presentation, and outcome of Legionnaires' disease have proven that there are differences between patients with community-acquired and nosocomial legionellosis. Hospitalized patients experienced a more severe clinical course, more often had diabetes (35%) and severe hyponatremia (23%), and had a high mortality rate (18%). In contrast, outpatients were significantly younger, showed an equal sex distribution, had significantly fewer comorbidities, and invariably experienced an uncomplicated clinical course without fatalities (Von Baum et al. 2008). There are also differences between cases which occurred during an outbreak and sporadic ones. Research conducted by Sopena et al. showed that male sex, chronic lung disease, an HIV infection, and immunosuppressive therapy prevailed in sporadic cases (Sopena et al. 2007). Presentation with respiratory symptoms, confusion, hyponatremia, and blood urea nitrogen and aspartate aminotransferase elevation was more frequent in sporadic cases, while headache prevailed in outbreak cases. Sporadic cases had a greater delay in treatment, were more severe, and had a worse course than epidemic cases (Sopena et al. 2007).

RATES OF MORTALITY

Infection with *Legionella* species may produce significant morbidity and mortality if not recognized and treated correctly. Data from the USA and Australia show case-fatality rates of 14% for nosocomial infections and 5–10% for community-acquired infections (Benin et al. 2002; Howden et al. 2003). In Europe, the overall case-fatality rate is about 6.6% (Ricketts and Joseph 2007). However, the mortality rate increases significantly in patients with underlying health problems. Multivariate analyses applied to patients under 60 years old revealed that cancer or hemopathy, underlying renal disease, or alcohol abuse were associated with the highest mortality. For older patients (>60 years) the factors related to mortality were cancer/hemopathy and renal or cardiac disease (Poupard et al. 2007).

Among the youngest patients, mortality was 33% and was higher among patients with hospital-acquired infections (41%) and among children younger than 2 months old (42%) compared with community-acquired cases (23%). The mortality rate was extremely high among patients treated with inappropriate antibiotics (76%) compared with patients treated appropriately (24%) (Greenberg 2005). The high mortality rates, especially in immunocompromised individuals, illustrate the fact that pneumonia caused by *Legionella* is still a challenging infectious disease.

TREATMENT OF LEGIONNAIRES' DISEASE

Early initiation of an appropriate treatment is crucial for a successful outcome of Legionnaires' disease. A prompt specific therapy reduces the fatality rate by 2- to 6-fold. *Legionella* spp. are intracellular pathogens; thus antibiotics with adequate intracellular penetration into macrophages are more likely to be efficacious against these bacteria. Antibiotics that fulfill this criterion include macrolides, fluoroquinolones, tetracyclines, rifamycins, and ketolides. Erythromycin was the original macrolide used after the first outbreak of Legionnaires' disease (Fraser et al. 1977); however, laboratory studies indicate that fluoroquinolones and the newer macrolides have a greater activity and better intracellular penetration than erythromycin (Garrido et al. 2005). Of the macrolides, azithromycin has been reported to be superior to clarithromycin or erythromycin (Fitzgeorge et al. 1993). Of the fluoroquinolones, levofloxacin and gemifloxacin appear to be the most active. MIC90 values against 85 isolates of *L. pneumophila* serogroup 1 ranged from 0.016 mg/l for levofloxacin, moxifloxacin, grepafloxacin, and gemifloxacin to 0.03 mg/l for ciprofloxacin and ofloxacin (Dubois and St-Pierre 2000; Yoo et al. 2004).

Fluoroquinolones have been recommended for transplant recipients with Legionnaires' disease because, unlike macrolides and rifampicin, they do not interfere with the metabolism of immunosuppressive medications (Stout and Yu 1997). In addition, patients with severe cases of the disease treated with fluoroquinolones have had a shorter hospital stay, a shorter time to apyrexia, and a decreased rate of complications compared with patients treated with macrolides (Garrido et al. 2005; Sabria et al. 2005). Ofloxacin and levofloxacin have been successfully used in the treatment of Legionnaires' disease despite heavy immunosuppression in some of the patients (Pedro-Botet et al. 2003; Sabria and Campins 2003). In spite of their high efficiency, however, respiratory fluoroquinolones relatively frequently cause adverse effects, such as gastrointestinal symptoms and central nervous system disturbances. Alteration of the electrocardiogram precludes the use of these quinolones in patients with severe electrolyte imbalances, irregular heartbeats, or severe congestive heart failure (Bartram et al. 2007).

Comparative studies of agents showing activity against atypical pathogens (macrolides, fluoroquinolones, or tetracyclines) versus β-lactams did not demonstrate significant differences in survival and clinical efficacy of patients receiving either treatment. However, the agents against atypical pathogens proved beneficial in the treatment of infections caused by *L. pneumophila* (Bartlett 2008).

In children, macrolides, especially azithromycin, are an effective and well-tolerated first-line treatment (Esposito et al. 2005; Langtry and Balfour 1998). Erythromycin and rifampicin have been the recommended antimicrobial therapeutic agents in the pedi-

atric population; however, some patients have failed to respond to these antibiotics, administered either singly or in combination (Brady 1989). Fluoroquinolones have proved to be very effective in the treatment of Legionnaires' disease in immunocompromised children, but their use in patients younger than 18 years old is, on the whole, contraindicated since it is linked to cartilage damage in juvenile animals (Gonzalez and Martin 2007).

The response to treatment depends on the patient's age, immune status, degree of pulmonary involvement, and the timing of the treatment. In mild to moderate cases in immunocompetent persons with a rapid response to therapy, a duration of 10 days is sufficient (Roig and Rello 2003). In immunocompromised individuals and/or those with severe disease, a 3-week treatment with either fluoroquinolones or macrolides (other than azithromycin) is necessary to avoid a relapse (Roig and Rello 2003). Patients with endocarditis or cavitating pneumonia may require longer courses of therapy.

During the treatment, attention should be paid to the possibility that in some cases one or more additional organisms may be involved, resulting in a polymicrobial infection. The culture of these co-infectors has revealed a wide spectrum of organisms, including bacteria such as *Streptococcus pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Listeria monocytogenes* as well as viruses and fungi (Roig and Rello 2003). A mixed infection is a risk factor for more severe Legionnaires' disease (Guiterrez et al. 2005).

PREVENTION

Legionnaires' disease is always transmitted from the environment to humans and thus constitutes an environmental disease that cannot be transmitted from person to person. Hence environmental monitoring, especially of potable water, cooling towers, and related sources, has become a major focus in efforts to control the spread of this disease. Special effort should be made in places where people at higher risk dwell or are treated (transplant patients, patients in immunosuppression or treated with corticosteroids). It is crucial to abide by hygienic-technical rules while planning and constructing water supply equipment in buildings.

REFERENCES

Adeleke AA, Fields BS, Benson RF et al (2001) *Legionella drozanski* sp. nov., *Legionella rowbothamii* sp. nov. and *Legionella fallonii* sp. nov: tree unusual new *Legionella* species. Int J Syst Evol Microbiol 51:1151–1160

Adeleke AA, Pruckler JM, Benson RF et al (1996) *Legionella*-like amebal pathogens – phylogenetic status and possible role in respiratory disease. Emerg Infect Dis 2:225–230

Akyildiz B, Gümüs H, Kumandas S et al (2008) Guillain-Barre syndrome associated with Legionella infection. J Trop Pediatr 54:275–277

Andereya S, Schneider U, Siebert C et al (2004) Reactive knee and ankle joint arthritis: abnormal manifestation of *Legionella pneumophila*. Rheumatol Int 24:182–184

Bartlett JG (2008) Is activity against "atypical" pathogens necessary in the treatment protocols for community-acquired pneumonia? Issues with combination therapy. Clin Infect Dis 47(suppl 3):S232–236.

Bartram J, Chartier Y, Lee JV et al (2007) Legionella and the prevention of legionellosis. WHO Press World Health Organization, Geneva, Switzerland

Benin AL, Benson RF, Arnold KL et al (2002) An outbreak of travel-associated Legionnaires' disease and Pontiac fever: the need for enhanced surveillance of travel-associated legionellosis in the United States. J Infect Dis 185:237–243

Borchardt J, Helbig JH, Lück PC (2008) Occurrence and distribution of sequence types among *Legionella pneumophila* strains isolated from patients in Germany: common features and differences to other regions of the world. Eur J Clin Microbiol Infect Dis 27:29–36

Brady MT (1989) Nosocomial legionnaires' disease in children's hospital. J Pediatr 115:46–50

Breiman RF, Butler JC (1998) Legionnaires' disease: Clinical, epidemiological, and public health perspectives. Semin Respir Infect 13:84–89

Brenner DJ, Krieg NR, Staley JT et al (2005) *Legionellales*. In: Bergey's Manual of Systematic Bacteriology, vol 2 (The Proteobacteria), part B (The Gammaproteobacteria), Springer, New York, p 210

Burnsed LJ, Hicks LA, Smithee LM et al (2007) A large travel-associated outbreak of legionellosis among hotel guests: utility of the urine antigen assay in confirming Pontiac fever. Clin Infect Dis 44:222–228

Campins M, Ferrer A, Callis L et al (2000) Nosocomial Legionnaire's disease in a children's hospital. Pediatr Infect Dis J 19:228–234

Casaulta Aebischer C, Matter L, Gaia V et al (1999) Diagnosis by polymerase chain reaction of pneumonia caused by *Legionella pneumophila* in an immunocompetent child. Infection 27:280–282

Castor ML, Wagstrom EA, Danila RN et al (2005) An outbreak of Pontiac fever with respiratory distress among workers performing high-pressure cleaning at a sugar-beet processing plant. J Infect Dis 191:1530–1537

Chaisson RE (1998) Bacterial pneumonia in patients with human immunodeficiency virus infection. Semin Respir Infect 4:133–138

Chown JW, Yu VL (1998) Legionella: a major opportunistic pathogen in transplant recipients. Semin Respir Infect 13:132–139

Cianciotto NP (2007) Iron acquisition by *Legionella pneumophila*. Biometals 20:323–331

Cunha BA (2005a) Diagnostic significance of non-specific laboratory tests in infectious diseases. In: Gorbach SL, Bartlett JB,

Blacklow NR (eds) Infectious diseases in medicine and surgery, 3rd edn. W. B. Saunders Co., Philadelphia, pp 158–165

Cunha BA (2005b) Elevated serum transaminases in *Mycoplasma pneumoniae* pneumonia. Clin Microbiol Infect 11:1051–1054

Cunha BA (2006a) The atypical pneumonias: clinical diagnosis and importance. Clin Microbiol Infect 12:12–24

Cunha BA (2006b) Hypophosphatemia: diagnostic significance in Legionnaires' disease. Am J Med 119:e5–6

Cunha BA (2008a) Atypical pneumonias: current clinical concepts focusing on Legionnaires' disease. Curr Opin Pulm Med 14:183–194

Cunha BA (2008b) Severe *Legionella* pneumonia: rapid presumptive clinical diagnosis with Winthrop-University Hospital's weighted point score system (modified). Heart Lung 37:311–320

Cunha BA (2008c) Highly elevated serum ferritin levels as a diagnostic marker for Legionella pneumonia. Clin Infect Dis 46:1789–1791

Del Pozo JL (2008) Update and actual trends on bacterial infections following liver transplantation. World J Gastroenterol 28:4977–4983

Den Boer JW, Yzerman EP (2004) Diagnosis of *Legionella* infection in Legionnaires' disease. Eur J Clin Microbiol Infect Dis 23:871–878

Den Boer JW, Yzerman EP, Schellekens J et al (2002) A large outbreak of Legionnaires' disease at a Dutch flower show. Emerg Infect Dis 8:37–43

Dennis PJ, Lee JV (1988) Differences in aerosol survival between pathogenic and non-pathogenic strains of *Legionella pneumophila* serogroup 1. J Appl Bacteriol 65:135–141

Di Stefano F, Verna N, Di Gioacchino M (2007) Cavitary *Legionella* pneumonia in a patient with immunodeficiency due to Hyper-IgE syndrome. J Infect 54:e121–e123

Diederen BM (2008) *Legionella* spp. and Legionnaires' disease. J Infect 56:1–12

Dournon E, Bibb WF, Rjagopalan P et al (1988) Monoclonal antibody reactivity as a virulence marker for *Legionella pneumophila* serogroup 1 strains. J Infect Dis 157:496–501

Drenning SD, Stout JE, Joly JR et al (2001) Unexpected similarity of pulsed-field gel electrophoresis patterns of unrelated clinical isolates of *Legionella pneumophila*, serogroup 1. J Infect Dis 183:628–632

Dubois J, St-Pierre C (2000) Comparative *in vitro* activity and post-antibiotic effect of gemifloxacin against *Legionella* spp. J Antimicrob Chemother 45(suppl):41–46

Edelstein PH (2007) Urine antigen tests positive for Pontiac fever: implications for diagnosis and pathogenesis. Clin Infect Dis 44:229–231

Eisenberg VH, Eidelman LA, Arbel R et al (1997) Legionnaires' disease during pregnancy: a case presentation and review of the literature. Eur J Obstet Gynecol Reprod Biol 72:15–18

Eitrem R, Forsgren A, Nilsson C (1987) Pneumonia and acute pancreatitis most probably caused by a *Legionella longbeachae* infection. Scand J Infect Dis 19:381–382

Esposito AL, Gantz MN (1986) Legionnaires' disease: the distance travelled since Philadelphia. Pediatr Infect Dis J 5:163–166

Esposito S, Bosis S, Faelli N et al (2005) Role of atypical bacteria and azithromycin therapy for children with recurrent respiratory tract infections. Pediatr Infect Dis J 24:438–444

Euzeby JP (2008) List of bacterial names with standing in nomenclature: http://www.bacterio.cict.fr/

Falguera M, Sacristan O, Nogues A et al (2001) Nonsevere community-acquired pneumonia. Correlation between cause and severity or comorbidity. Arch Intern Med 161:1866–1872

Famiglietti RF, Bakerman PR, Saubolle MA et al (1997) Legionellosis in two immunocompetent infants. Pediatrics 99:899–903

Fang GD, Yu VL, Vickers RM (1989) Disease due to the *Legionellaceae* (other than *Legionella pneumophila*): Historical, microbiological, clinical, and epidemiological review. Medicine 68:116–132

Faris B, Faris C, Schousboe M et al (2005) Legionellosis from *Legionella pneumophila* serogroup 13. Emerg Infect Dis 11:1405–1409

Fields BS, Benson RF, Besser RE (2002) Legionella and Legionnaires' disease: 25 years of investigation. Clin Microbiol Rev 15:506–526

Fitzgeorge RB, Lever S, Baskerville A (1993) A comparison of the efficacy of azithromycin and clarithromycin in oral therapy of experimental airborne legionnaires' disease. J Antimicrob Chemother 31(suppl E):171–176

Franzin L, Cabodi D, Scolfaro C et al (2004) Microbiological investigations on a nosocomial case of *Legionella pneumophila* pneumonia associated with water birth and review of neonatal cases. Infez Med 12:69–75

Franzin L, Scolfaro C, Cabodi D et al (2001) *Legionella pneumophila* pneumonia in a newborn after water birth: a new mode of transmission. Clin Infect Dis 33:103–104

Fraser DW, Tsai TR, Orenstein W et al (1977) Legionnaires' disease – description of an epidemic of pneumonia. N Engl J Med 297:1189–1197

Fry NK, Bangsborg JM, Bergmans A et al (2002) Designation of the European Working Group on Legionella Infections (EWGLI) amplified fragment length polymorphism types of *Legionella pneumophila* serogroup 1 and results of intercentre proficiency testing using a standard protocol. Eur J Clin Microbiol Infect Dis 21:722–728

Fry NK, Rowbotham TJ, Saunders NA et al (1991) Direct amplification and sequencing of the 16S ribosomal DNA of an intracellular *Legionella* species recovered by amebal enrichment from the sputum of a patient with pneumonia. FEMS Microbiol Lett 83:165–168

Furugen M, Koide M, Baba M et al (2008) Legionella pneumonia caused by *Legionella pneumophila* serogroup 2: second case report in Japan. J Infect Chemother 14:161–165

Gaillac N, Floccard B, Ould T et al (2006) *Legionella pneumophila* pneumonia during pregnancy: a case report. J Infect 52:e163–164

Garcia-Vidal C, Carratala J (2006) Current clinical manage-

ment of Legionnaires' disease. Expert Rev Anti Infect Ther 4:995–1004

Garrido RM, Parra FJ, Frances LA et al (2005) Antimicrobial chemotherapy for Legionnaires' disease: levofloxacin versus macrolides. Clin Infect Dis 40:800–806

Gonzalez IA, Martin JM (2007) Legionella pneumophila serogroup 1 pneumonia recurrence postbone marrow transplantation. Pediatr Infect Dis J 26:961–963

Granados A, Podzamczer D, Gudiol F et al (1989) Pneumonia due to *Legionella pneumophila* and pneumococcal pneumonia: similarities and differences on presentation. Eur Respir J 2:130–134

Greenberg D (2005) Legionnaires' disease in children: is it an emerging or an under-diagnosed disease? ASM 45th General Meeting, Washington D.C., Poster G-820

Greenberg D, Chiou CC, Famigilleti R et al (2006) Problem pathogens: paediatric legionellosis-implications for improved diagnosis. Lancet Infect Dis 6:529–535

Guillouzouic A, Bemer P, Gay-Andrieu F et al (2008) Fatal coinfection with *Legionella* serogroup 8 and *Aspergillus fumigatus*. Diagn Microbiol Infect Dis 60:193–195

Gupta SK, Imperiale TF, Sarosi GA (2001) Evaluation of the Winthrop-University Hospital. Criteria to identify *Legionella* pneumonia. Chest 120:1064–171

Gutierrez F, Masia M, Rodriguez JC et al (2005) Community--acquired pneumonia of mixed etiology, prevalence and outcome. Eur J Clin Microbiol Infect Dis 24:377–383

Gutierrez Rodero F, Ortiz de la Tabla V, Martinez C et al (1995) Legionnaires' disease in patients infected with human immunodeficiency virus. Clin Infect Dis 21:712–713

Guyot S, Goy JJ, Gersbach P et al (2007) *Legionella pneumophila* aortitis in a heart transplant recipient. Transpl Infect Dis 9:58–59

Haeuptle J, Zaborsky R, Fiumefreddo R et al (2009) Prognostic value of procalcitonin in *Legionella* pneumonia. Eur J Clin Microbiol Infect Dis 28:55–60

Haluk E, Aynur Y, Oznur K et al (2006) Rhabdomyolysis--induced acute renal failure associated with legionnaires' disease. Scan J Urol Nephrol 40:345–346

Harrison TG, Doshi N, Fry NK et al (2007) Comparison of clinical and environmental isolates of *Legionella pneumophila* obtained in the UK over 19 years. Clin Microbiol Infect 13:78–85

Harrison TG, Fry NK, Afshar B et al (2006) Typing of *Legionella pneumophila* and its role in elucidating the epidemiology of Legionnaires' disease. In: Cianciotto NP, Kwaik Y, Edelstein PS et al (eds) *Legionella*: state of the art 30 years after its recognition. Washington, D. C.: American Society for Microbiology, 94–99

Helbig JH, Bernander S, Castellanii Pastoris M et al (2002) Pan-European study on culture-proven Legionnaires' disease: distribution of *Legionella pneumophila* serogroups and monoclonal subgroups. Eur J Clin Microbiol Infect Dis 21:710–716

Holmberg RE, Pavia AT, Montgomery D et al (1993) Nosocomial *Legionella* pneumonia in the neonate. Pediatrics 92:450–453

Horbach I, Fehrenbach FJ (1990) Legionellosis in heart transplant recipients. Infection 18:361–363

Howden BP, Stuart RL, Tallis G et al (2003) Treatment and outcome of 104 hospitalized patients with Legionnaires' disease. Intern Med J 33:484–488

Imai N, Yagi N, Konishi T et al (2008) Legionnaires' disease with hypoperfusion in the cerebellum and frontal lobe on single photon emission computed tomography. Intern Med 47:1263–1266

Jacobson KL, Miceli MH, Tarrand JJ et al (2008) Legionella pneumonia in cancer patients. Medicine 87:152–159

Jeon TJ, Jeon KW (2004) Gene switching in *Amoeba proteus* caused by endosymbiotic bacteria. J Cell Sci 117:535–543

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

Joly JR, McKinney RM, Tobin JO et al (1986) Development of a standardized subgrouping scheme for *Legionella pneumophila* serogroup 1 using monoclonal antibodies. J Clin Microbiol 23:768–771

Joseph CA (2004) Legionnaires' disease in Europe 2000–2002. Epidemiol Infect 132:417–424.

Joseph CA, Watson JM, Harrison TG et al (1994) Nosocomial Legionnaires' disease in England and Wales, 1980–92. Epidemiol Infect 112:329–345

Karim A, Ahmed S, Rossoff LJ (2002) Legionnaires' disease associated with acute encephalitis and arrhythmia. Crit Care Med 30:1028–1029

Kim KW, Goo JM, Lee HJ et al (2007) Chest computed tomographic findings and clinical features of Legionella pneumonia. J Comput Assist Tomogr 31:950–955

König C, Hebestreit H, Valenza G et al (2005) *Legionella waltersii* – a novel cause of pneumonia? Acta Paediatr 94:1505–1507

Kümpers P, Tiede A, Kirschner P et al (2008) Legionnaires' disease in immunocompromised patients: a case report of *Legionella longbeachae* pneumonia and review of the literature. J Med Microbiol 57:384–387

La Scola B, Birtles RJ, Greub G et al (2004) *Legionella drancourtii* sp. nov., a strictly intracellular amoebal pathogen. Int J Syst Evol Microbiol 54:699–703

La Scola B, Maltezou H (2004) *Legionella* and Q fever community acquired pneumonia in children. Pediatr Respir Rev 5(suppl A):S171–S177

Langtry HD, Balfour JA (1998) Azithromycin. A review of its use in paediatric infectious diseases. Drugs 56:273–297

Lepine LA, Jernigan DB, Butler JC et al (1998) A recurrent outbreak of nosocomial Legionnaire's disease detected by urinary antigen testing: evidence for long-term colonization of a hospital plumbing system. Infect Control Hosp Epidemiol 19:905–910

Lettinga KD, Verbon A, Nieuwkerk PT et al (2002a) Health--related quality of life and posttraumatic stress disorder among survivors of an outbreak of legionnaires' disease. Clin Infect Dis 35:11–17

Lettinga KD, Verbon A, Weverling GJ et al (2002b) Legionnaires' disease at a Dutch flower show: prognostic factors and impact of therapy. Emerg Infect Dis 8:1448–1454

Levy I, Rubin LG (1998) Legionella pneumonia in neonates: a literature review. J Perinatol 18:287–290

Levy PY, Teysseire N, Etienne J et al (2003) A nosocomial outbreak of *Legionella pneumophila* caused by contaminated transesophageal echocardiography probes. Infect Control Hosp Epiemiol 24:619–622

Lück PC, Dinger E, Helbig JH et al (1994) Analysis of *Legionella pneumophila* strains associated with nosocomial pneumonia in a neonatal intensive care unit. Eur J Clin Microbiol Infect Dis 13:565–571

Lück PC, Schneider T, Wagner J et al (2008) Community--acquired Legionnaires' disease caused by *Legionella pneumophila* serogroup 10 linked to the private home. J Med Microbiol 57:240–243

Lüttichau HR Vinther C, Uldum SA et al (1997) An outbreak of Pontiac fever among children following use of a whirlpool. Clin Infect Dis 26:1374–1378

Maltezou H, La Scola B, Astra H et al (2004) Mycoplasma pneumoniae and Legionella pneumophila in community-acquired lower respiratory tract infections among hospitalized children: diagnosis by real time PCR. Scand J Infect Dis 36:639–642

Marston B, Lipman HB, Brieman RF (1994) Surveillance for legionnaires' disease. Risk factors for morbidity and mortality. Arch Intern Med 154:2417–2422

Marston BJ, Plouffe JF, File TJ et al (1997) Incidence of community-acquired pneumonia requiring hospitalizations: results of a population-based active surveillance study in Ohio. The Community-Based Pneumonia Incidence Study Group. Arch Intern Med 157:1709–1718

Masia M, Gutierrez F, Padilla S et al (2007) Clinical characterization of pneumonia caused by atypical pathogens combining classic and novel predictors. Clin Microbiol Infect 13:153–161

Mathys W, Deng MC, Meyer J et al (1999) Fatal nosocomial Legionnaires' disease after heart transplantation: clinical course, epidemiology and prevention strategies for the highly compromised host. J Hosp Infect 43:242–246

McClelland MR, Vaszar LT, Kagawa F (2004) Pneumonia and osteomyelitis due to *Legionella longbeachae* in a woman with systemic lupus erythematosus. Clin Infect Dis 38:102–106

McNally C, Hackman B, Fields BS et al (2000) Potential importance of *Legionella* species as etiologies in community acquired pneumonia (CAP). Diagn Microbiol Infect Dis 38:79–82

Miller ML, Hayden R, Gaur A (2007) Legionella bozemanii pulmonary abscess in a pediatric allogeneic stem cell transplant recipient. Pediatr Infect Dis J 26:760–762

Mrozińska M (2005) Legionellosis in three children – cases report. Przegl Epidemiol 59:851–857

Muder RR, Yu VL (2002) Infection due to *Legionella* species other than *L. pneumophila*. Clin Infect Dis 35:990–998

Nagai T, Sobajima H, Iwasa M (2000) A fatal newborn case of *Legionella pneumophila* pneumonia occurring after water birth in a bathtub with an all day circulating system. IASR 13:190–191

Neil K, Berkelman R (2008) Increasing incidence of legionellosis in the United States, 1990–2005: changing epidemiologic trends. Clin Infect Dis 47:591–599

O'Connor BA, Carman J, Eckert K et al (2007) Does using potting mix make you sick? Results from a *Legionella longbeachae* case-control study in South Australia. Epidemiol Infect 135:34–39

Orenstein WA, Overturf GD, Leedom JM et al (1981) The frequency of *Legionella* infection prospectively determined in children hospitalized with pneumonia. J Pediatr 99:403–406

Pai P, Kumar S, Bell GM et al (1996) Rapidly progressive crescentic glomerulonephritis and Legionnaires' disease. Clin Nephrol 45:209–210

Paradkar PN, De Domenico I, Durchfort N et al (2008) Iron depletion limits intracellular bacterial growth in macrophages. Blood 112:866–874

Park M, Yun ST, Kim MS et al (2004) Phylogenetic characterization of *Legionella*-like endosymbiotic X-bacteria in *Amoeba proteus*: a proposal for "Candidatus *Legionella jeonii*" sp. nov. Environ Microbiol 6:1252–1263

Pedro-Botet ML, Sabria M, Sopena N et al (2003) Legionnaires' disease and HIV infection. Chest 124:543–547

Phares CR, Wangroonngsarb P, Chantra S et al (2007) Epidemiology of severe pneumonia caused by *Legionella longbeachae*, *Mycoplasma pneumonia*, and *Chlamydia pneumonia*: 1-year, population-based surveillance for severe pneumonia in Thailand. Clin Infect Dis 45:147–155

Poupard M, Campese C, Bernillon P et al (2007) Factors associated with mortality in Legionnaires' disease, France, 2002–2004. Med Mal Infect 37:325–330

Qin X, Abe PM, Weissman SJ et al (2002) Extrapulmonary *Legionella micdadei* infection in a previously healthy child. Pediatr Infect Dis J 21:1174–1176

Quagliano PV, Das Narla L (1993) *Legionella* pneumonia causing multiple cavitating pulmonary nodules in a 7-month--old infant. AJR Am J Roentgenol 161:367–368

Redd SC, Schuster DM, Quan J et al (1988) Legionellosis in cardiac transplant recipients: results of a nationwide survey. J Infect Dis 158:651–653

Ricketts KD, Joseph CA (2007) Legionnaires' disease in Europe 2005–2006. Euro Surveill 12:E7–8

Roig J, Aguilar X, Ruiz J et al (1991) Comparative study of *Legionella pneumophila* and other nosocomial-acquired pneumonias. Chest 99:344–350

Roig J, Rello J (2003) Legionnaires' disease: a rational approach to therapy. J Antimicrob Chemother 51:1119–1129

Rowbotham TJ (1993) *Legionella*-like amoebal pathogens. In: Barbaree JM, Breiman RF, Dofour AP (eds) Legionella – current status and emerging perspectives. ASM Press, Washington, pp 137–140

Ryan ME, Feldman S, Pruitt B et al (1979) Legionnaires' disease in a child with cancer. Pediatrics 64:951–953

Sabria M, Campins M (2003) Legionnaires' disease: update on

epidemiology and management options. Am J Respir Med 2:235–243

Sabria M, Pedro-Botet ML, Gomez J et al (2005) Fluoroquinolones vs macrolides in the treatment of Legionnaires disease. Chest 128:1401–1405

Sakai F, Tokuda H, Goto H et al (2007) Computed tomographic features of Legionella pneumophila pneumonia in 38 cases. J Comput Assist Tomogr 31:125–131

Sandkovsky U, Sandkovsky G, Suh J et al (2008) Legionella pneumonia and HIV: case reports and review of the literature. AIDS Patient Care STDS 22:473–481

Saunders NA, Harrison TG, Haththotuwa A et al (1990) A method for typing strains of *Legionella pneumophila* serogroup 1 by analysis of restriction fragment length polymorphisms. J Med Microbiol 31:45–55

Scaturro M, Losardo M, De Ponte G et al (2005) Comparison of the free molecular methods used for subtyping of *Legionella pneumophila* strains isolated during an epidemic of Legionellosis in Rome. J Clin Microbiol 43:5348–5350

Shelburne SA, Kielhofner MA, Tiwari PS (2004) Cerebellar involvement in legionellosis. South Med J 97:61–64

Shimura C, Saraya T, Wada H et al (2008) Pathological evidence of rhabdomyolysis-induced acute tubulointerstitial nephritis accompanying *Legionella pneumonia* pneumonia. J Clin Pathol 61:1062–1063

Singh N, Gayowski T, Wagener M et al (1996) Pulmonary infections in liver transplant recipients receiving tacrolimus. Changing pattern of microbial etiologies. Transplantation 61:396–401

Singh N, Stout J, Yu VL (2002) Legionnaires' disease in a transplant recipient acquired from the patient's home: implications for management. Transplantation 74:755–756

Sopena N, Force L, Pedro-Botet ML et al (2007) Sporadic and epidemic community legionellosis: two faces of the same illness. Eur Respir J 29:138–142

Sopena N, Martinez-Vazquez C, Rodriguez-Suarez JR et al (1998) Comparative study of the clinical presentation of *Legionella* pneumonia and other community-acquired pneumonias. Chest 113:1195–200

Stout JE, Yu VL (1997) Legionellosis. N Engl J Med 337:682–687

Sugihara E, Dambara T, Aiba M et al (2007) Clinical characteristics of 8 sporadic cases of community-acquired Legionella pneumonia in advanced age. Intern Med 46:461–465

Takunaga Y, Concepcion W, Berquist WE et al (1992) Graft involvement by Legionella in a liver transplant recipient. Arch Surg 127:475–477

Tewari K, Wold SM, Asrat T (1997) Septic shock associated with *Legionella* pneumonia: case report. Am J Obstet Gynecol 176:706–707

Tossa P, Deloge-Abarkan M, Zmirou-Navier D et al (2006) Pontiac fever: an operational definition for epidemiological studies. BMC Public Health 6:112

Trübel HK, Meyer HG, Jahn B et al (2002) Complicated nosocomial pneumonia due to *Legionella pneumophila* in immunocompromised child. Scand J Infect Dis 34:219–221

Venezia RA, Hanley EM, Urquhart K et al (1994) Nosocomial legionellosis associated with aspiration of nasogastric feedings diluted in tap water. Infect Control Hosp Epidemiol 15:529–533

Vergis EN, Akbas E, Yu VL (2000) *Legionella* as a cause of severe pneumonia. Semin Respir Crit Care Med 21:295–304

Vimercati A, Greco P, Bettocchi S et al (2000) Legionnaire's disease complicating pregnancy: a case report with intrauterine fetal demise. J Perinat Med 28:147–150

Von Baum H, Ewig S, Marre R et al (2008) Community--acquired *Legionella* pneumonia: new insights for the German competence network for community-acquired pneumonia. Clin Infect Dis 46:1356–1364

Watkins ID, Tobin JO, Dennis PJ et al (1985) *Legionella pneumophila* serogroup 1 subgrouping by monoclonal antibodies – an epidemiological tool. J Hyg 95:211–216

Watson AM, Boyce TG, Wylam ME (2004) Legionella pneumonia: infection during immunosuppressive therapy for idiopathic pulmonary hemosiderosis. Pediatr Infect Dis J 23:82–84

Wondergem MJ, Voskuyl AE, van Agtmael MA (2004) A case of Legionellosis during treatment with a TNF-α antagonist. Scand J Infect Dis 36:310–311

Woodhead M (2002) Community-acquired pneumonia in Europe: causative pathogens and resistance patterns. Eur Respir J Suppl 36:20s–27s

Yoo BK, Triller DM, Yong CS (2004) Gemifloxacin: a new fluoroquinolone approved for treatment of respiratory infections. Ann Pharmacother 38:1226–1235

Yu VL, Plouffe JF, Castellanii-Pastoris M et al (2002) Distribution of *Legionella* species and serogroups isolated by culture in patients with sporadic community-acquired legionellosis: an international collaborative survey. J Infect Dis 186:127–128