

Infection Due to *Legionella* Species Other Than *L. pneumophila*

Robert R. Muder and Victor L. Yu

Infectious Diseases Section, VA Pittsburgh Healthcare System and University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

In addition to *Legionella pneumophila*, 19 *Legionella* species have been documented as human pathogens on the basis of their isolation from clinical material. Like *L. pneumophila*, other *Legionella* species are inhabitants of natural and man-made aqueous environments. The major clinical manifestation of infection due to *Legionella* species is pneumonia, although nonpneumonic legionellosis (Pontiac fever) and extrapulmonary infection may occur. The majority of confirmed infections involving non-*pneumophila* *Legionella* species have occurred in immunosuppressed patients. Definitive diagnosis requires culture on selective media. Fluoroquinolones and newer macrolides are effective therapy. A number of nosocomial cases have occurred in association with colonization of hospital water systems; elimination of *Legionella* species from such systems prevents their transmission to susceptible patients. It is likely that many cases of both community-acquired and nosocomial *Legionella* infection remain undiagnosed. Application of appropriate culture methodology to the etiologic diagnosis of pneumonia is needed to further define the role of these organisms in disease in humans.

Although 90% of *Legionella* infections in humans are caused by *Legionella pneumophila*, there are 45 named species of *Legionella* [1, 2]. Like *L. pneumophila*, these species inhabit a wide variety of naturally occurring and man-made environments, including water [3, 4], soil [5], and water distribution systems [6]. A number of *Legionella* species are primary pathogens of free-living amoebae. Additional strains of bacteria that infect amoebae, known as “*Legionella*-like amebal pathogens” (LLAP), appear to be closely related to *Legionella* species on the basis of 16S rRNA gene sequencing [7–9]. Several of these strains have been assigned to the *Legionella* genus [2]. One of them, LLAP-3, was first isolated from the sputum of a patient with pneumonia after the clinical specimen was incubated with *Acanthamoeba polyphaga* [10]. Serologic surveys of patients with community-acquired pneumonia have suggested that LLAP may be occasional human pathogens [10, 11]. *Legionella* species are widespread in water and soil; humans are incidental hosts that acquire the organisms after exposure to natural or

man-made aquatic reservoirs. In addition to *L. pneumophila*, 19 species are documented as human pathogens on the basis of their isolation from clinical material [12–31].

MICROBIOLOGY

Legionellae are fastidious gram-negative aerobic bacilli. They do not grow on most standard media used for primary isolation of respiratory pathogens in the clinical microbiology laboratory. Legionellae will grow on buffered charcoal yeast extract agar; however, several species grow poorly on this medium [32]. Members of the genus share several phenotypic features, including catalase and oxidase activity, and they require cysteine for growth. They are nonfermentative as well as urease and nitrate negative. Legionellae produce a water-soluble extracellular compound that fluoresces yellow-green on exposure to ultraviolet light; several species exhibit intracellular red, white, or blue autofluorescence. In general, phenotypic characteristics are of limited usefulness in differentiating species. In the clinical microbiology laboratory, direct fluorescent antibody (DFA) testing of isolates permits rapid identification of species. Analysis of 16S rRNA, ubiquinone contents, or cell-wall fatty acid profiles are reliable methods of speciation [7, 33], but these generally are not available to clinical laboratories.

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Reprints or correspondence: Dr. Robert R. Muder, Infectious Disease Section, VA Pittsburgh Healthcare System, University Dr. C, Pittsburgh, PA 15240 (Robert.Muder@med.va.gov).

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Table 1. Percentage of patients with pneumonia due to selected *Legionella* species who had an underlying medical condition or received immunosuppressive therapy.

<i>Legionella</i> species	Solid tumor	Hematologic malignancy	Organ transplant	Immunosuppressive therapy ^a
<i>L. micdadei</i> (n = 95)	14	19	27	72
<i>L. bozemanii</i> (n = 21)	10	24	24	76
<i>L. dumoffii</i> (n = 17)	12	6	6	38

NOTE. Data from [52].

^a Corticosteroids or cytotoxic chemotherapy.

EPIDEMIOLOGY

L. pneumophila and other *Legionella* species are found in similar environments; it is not unusual to recover >1 species from a given site. Legionellae occur naturally in a wide variety of aquatic habitats and in soil. A variety of man-made environments commonly harbor *Legionella* species; these include water distribution systems of buildings [6, 34, 35], cooling towers [36, 37], industrial equipment [38], and whirlpool spas [39]. *L. pneumophila* is the species most frequently isolated from water distribution systems, but *Legionella micdadei*, *Legionella bozemanii*, *Legionella dumoffii*, *Legionella anisa*, and *Legionella feeleii* are isolated relatively frequently [40–45]. These species may be more difficult to recover from water than is *L. pneumophila*, and, thus, their true prevalence may be somewhat underestimated. There is evidence that some *Legionella* species proliferate less readily in the presence of the sediment and commensal microflora of water systems than does *L. pneumophila* [46]. Several species appear less able to proliferate within protozoa than do *L. pneumophila*. For example, most strains of *L. pneumophila* multiply rapidly within *Acanthamoeba* species; *L. micdadei* does not [47, 48]. The optimal detection of non-*pneumophila* *Legionella* species in potable water can be achieved by acid buffer pretreatment of swab specimens and plating on buffered charcoal yeast extract agar [49].

The majority of *Legionella* infections in humans are caused by *L. pneumophila*. A multinational study of community-acquired legionnaires disease identified 508 culture-confirmed

cases [50]. *L. pneumophila* was responsible for the greatest percentage of cases (91.5%), followed by *Legionella longbeachae* (3.9%) and *L. bozemanii* (2.4%). The remainder of cases were due to *L. micdadei*, *L. feeleii*, *L. dumoffii*, *Legionella wadsworthii*, and *L. anisa*. The remaining *Legionella* species are rarely isolated from humans; only a single case of infection in a human has been reported in association with several of these species.

Patients with non-*pneumophila* *Legionella* infections are more likely to be immunocompromised as a result of receiving immunosuppressive therapy than are patients with *L. pneumophila* infection. During a concurrent outbreak of *L. pneumophila* pneumonia and *L. micdadei* pneumonia in a single institution, 53% of patients with *L. micdadei* infection were immunosuppressed as a result of hematologic malignancy or receipt of corticosteroid therapy or cancer chemotherapy, compared with 19% of those infected with *L. pneumophila* [51]. Table 1 lists the underlying medical conditions of patients with pneumonia due to selected *Legionella* species as reported by Fang et al. in 1989 [52]. A number of the cases that were reviewed were diagnosed by serologic means. Because serologic testing has not been validated for the diagnosis of infection due to *Legionella* species other than *L. pneumophila*, we reviewed cases of infection due to *L. micdadei* [6, 53–63], *L. bozemanii* [64–69], and *L. dumoffii* [45, 70–73] that were diagnosed by culture or DFA staining (table 2). The majority of infections occurred in immunocompromised patients, confirming the findings of previous reports. Nearly one-half of the

Table 2. Confirmation of infections due to *Legionella* species by culture or direct fluorescent antibody testing, 1989–2001 (literature review).

<i>Legionella</i> species	No. of infections documented by culture/total no. of infections	Underlying condition or receipt of therapy				
		Solid tumor	Hematologic malignancy	Organ transplant	HIV infection	Immunosuppressive therapy ^a
<i>L. micdadei</i>	25/35	17	14	51	6	11
<i>L. bozemanii</i>	9/10	0	10	30	30	20
<i>L. dumoffii</i>	8/8	0	12	38	0	12

NOTE. Data are the percentage of patients who had an underlying condition or received immunosuppressive therapy.

^a Corticosteroids or cytotoxic chemotherapy.

more recent infections occurred in transplant recipients; this may reflect the increasing use of transplantation.

It is not clear whether these non-*pneumophila* *Legionella* species are inherently less pathogenic than *L. pneumophila*. Many species are pathogenic for amoebae [74, 75] and share virulence genes with *L. pneumophila* [76]. The growth of *L. micdadei* within cultured human macrophages is generally less robust than that of *L. pneumophila*, but some strains of the former species show vigorous intracellular proliferation that is equal to that of *L. pneumophila* [77]. The relative infrequency of non-*pneumophila* *Legionella* species as causes of infection may be the result of their lower frequency and lesser concentrations in aquatic environments with which humans have contact. However, it should be noted that many infections with non-*pneumophila* *Legionella* species may be unrecognized because submission of sputum samples for *Legionella* culture is not a standard practice in the evaluation of community-acquired pneumonia. Furthermore, 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 due to *L. pneumophila* serogroup 1.

When specifically sought, unsuspected community-acquired infections may be uncovered. In a study of patients in Ohio who had community-acquired pneumonia of unknown etiology, 14% of patients showed seroconversion to a *Legionella* species, including *L. bozemanii* (8%) and *L. anisa* (4%) [78].

Legionella species are major opportunistic pathogens of solid-organ and bone marrow transplant recipients [6, 54, 60, 79, 80]. After *L. pneumophila*, *L. micdadei* and *L. bozemanii* are the next most common *Legionella* species to cause infection among transplant patients [80]. HIV-infected patients may have a somewhat increased risk for *Legionella* infection [81]. In addition to cases due to *L. pneumophila*, cases due to *L. bozemanii* [69], *L. micdadei* [61], and *L. feeleii* [82] have been reported in HIV-infected patients.

With few exceptions, reported clusters of pneumonia due to non-*pneumophila* *Legionella* species have been nosocomial. These nosocomial infections have included infections due to *L. micdadei* [6, 40, 53, 83], *L. bozemanii* [41], and *L. dumoffii* [42]. The source of infection in these outbreaks was the hospital water system, as is usually the case with nosocomial *L. pneumophila* infection. For example, an outbreak of nosocomial *L. micdadei* infection among heart and kidney transplant recipients involved 12 patients during a 6-month period at a single center [6]. The attack rate among transplant patients was 19%. *L. micdadei* was isolated from multiple sites in the hospital water system; environmental and clinical strains were identical by PFGE of bacterial DNA.

When >1 *Legionella* species is present in a hospital water system, outbreaks of pneumonia due to each species may occur concurrently; some patients may be infected with >1 species

simultaneously [84]. In one hospital, a cluster of cases of prosthetic valve endocarditis and a cluster of cases of postoperative sternal wound infection due to *L. pneumophila* and/or *L. dumoffii* were associated with the presence of these 2 species in the hospital water system [45, 85]. Bathing fresh postoperative wounds with contaminated tap water was the means of transmission for the postoperative outbreak of sternal wound infection.

Legionella species are occasional pathogens in nursing homes. Because most nursing home-acquired pneumonias are treated without a specific diagnosis being made, the true incidence of pneumonia due to *Legionella* species is unknown [86]. Intensive surveillance may identify unsuspected cases when *Legionella* species are present in the water system [87]. Two outbreaks of pneumonia due to *Legionella sainthelensi* occurred in Canadian nursing homes [34]. In 1 of these nursing homes, *L. sainthelensi* was isolated from the facility's water system.

Community outbreaks of pneumonia due to species other than *L. pneumophila* are uncommon; most cases appear to be sporadic. Exceptions to this generalization are community outbreaks of *L. longbeachae* pneumonia occurring in Australia [5] and on the West Coast of the United States [88]. The cases occurred in gardeners; commercially available potting soils were found to be colonized with *L. longbeachae*. The clinical isolates and isolates in soil that were recovered during the outbreak in Australia were found to be closely related on the basis of the results of restriction fragment-length polymorphism analysis [89]. The precise mode of transmission from soil is uncertain.

There are no reported outbreaks of pneumonia due to non-*pneumophila* *Legionella* species that have been associated with large aerosol-generating devices, such as cooling towers. However, there are numerous reported outbreaks of nonpneumonic legionellosis (Pontiac fever) associated with exposure to aerosols. These include an outbreak affecting >300 automobile plant workers exposed to an aerosol generated by machinery contaminated with *L. feeleii* [38]. Outbreaks of Pontiac fever due to *L. micdadei* and *L. anisa* have occurred in association with exposure to contaminated whirlpool spas [39] and decorative fountains [90].

CLINICAL ILLNESS

Pneumonia due to non-*pneumophila* *Legionella* species resembles that due to *L. pneumophila* clinically and radiographically [51, 52]. More than 90% of patients experience fever, and peak temperature exceeds 39.4°C (103°F) in ~50% of patients. Although most patients produce some sputum, its production is typically scant. Most patients will experience dyspnea. Alteration in mental status is often prominent; 60% of patients will experience a depression in their level of consciousness that ranges from lethargy to obtundation. However, *Legionella* in-

fection does not present with a distinctive clinical syndrome; it cannot be reliably differentiated from pneumonia due to other bacterial pathogens on the basis of signs and symptoms [91]. Patients receiving immunosuppressive agents may experience severe pleuritic chest pain, as is the case in immunosuppressed patients infected with *L. pneumophila*. The combination of dyspnea and pleuritic chest pain may lead to diagnostic confusion with pulmonary embolism [92, 93]. As in *L. pneumophila* infection, atypical presentations of pneumonia may occur in immunosuppressed patients. Fever and radiographic pulmonary infiltrates in the absence of respiratory symptoms have been reported [15, 51, 94], as have progressive pulmonary infiltrates in the absence of fever [15, 51, 94].

Radiographically, manifestations of pneumonia due to non-*pneumophila* *Legionella* species resemble those of pneumonia due to *L. pneumophila*. Segmental or lobar infiltrates are typical, particularly in nonimmunocompromised patients [95, 96]. Small to moderate pleural effusions are common. In immunocompromised patients, pulmonary infiltrates often are rounded or nodular [96]. In these patients, nodular infiltrates have a notable tendency to cavitate. The cavities often enlarge, in association with progressive thinning of the surrounding infiltrate. This may occur during therapy and in association with clinical improvement; specific intervention rarely is necessary. Cavitation of infiltrates appears to be related to the patients' underlying immune status rather than to the *Legionella* species involved; it is a very uncommon occurrence in patients with intact immune systems.

Laboratory findings do not distinguish *Legionella* pneumonia from that due to other bacteria. Most patients will demonstrate leukocytosis with a predominance of polymorphonuclear leukocytes; in patients receiving cytotoxic immunosuppressive or chemotherapeutic agents, leukocytosis may fail to develop. Mild elevations of transaminase levels and alkaline phosphatase are common. Hyponatremia occurs in approximately one-third of patients [97].

On the basis of reports in the literature, extrapulmonary infection appears to be rare. However, reports in the literature may underestimate the true frequency of extrapulmonary infection because clinical material from nonpulmonary infection is not likely to be cultured on media that will sustain the growth of *Legionella* species. Cutaneous abscess due to *L. micdadei* has been reported after pneumonia [98], most likely as a result of bacteremic seeding. There is one report of necrotizing cellulitis due to *L. micdadei* occurring in the absence of pulmonary infection [99]. A patient with nephrotic syndrome and monoclonal gammopathy had recurrent soft-tissue abscesses due to *Legionella cinцинnatiensis* develop at multiple sites [100]. There was no evidence of pneumonia, and the source of the infection was not determined.

A cluster of 4 cases of *L. dumoffii* prosthetic valve endocarditis occurred in a single hospital [85]; 1 patient was simul-

taneously infected with *L. pneumophila*. The infection presented as a chronic febrile illness with night sweats and weight loss; embolic phenomena were absent. Reported cases of sternal wound infection due to *L. pneumophila* presented as serosanguineous wound drainage after open-heart surgery. No organisms were identified by use of Gram stain, but *L. pneumophila* and *L. dumoffii* were isolated by culture on selective media [45]. There are reports of *L. bozemanii* and *L. dumoffii* pericarditis occurring in heart transplant recipients [64, 70].

Nonpneumonic legionellosis, or Pontiac fever, occurs after exposure to aerosols of water colonized with *Legionella* species [38, 39, 90]. Attack rates after exposure to an aerosol-generating source are exceptionally high, often in the range of 50% to 80%. After a typical asymptomatic interval of 12–48 h after exposure, patients note the abrupt onset of fever, chills, headache, malaise, and myalgias. Pneumonia is absent; those who are affected recover in 2–7 days without receiving specific treatment. Diagnosis is suggested by the occurrence of acute illness in multiple persons within a short time after exposure to a source of aerosols. Although patients show seroconversion to the implicated *Legionella* species, there is no evidence that *Legionella* infection occurs during the course of Pontiac fever. Indeed, the simultaneous occurrence of cases of pneumonia and outbreaks of Pontiac fever is quite unusual. The illness may be the result of an immunologic or hypersensitivity reaction to bacteria or their products; high levels of endotoxin in aerosolized water may be responsible for clinical symptoms [101].

DIAGNOSIS

Although *Legionella* species are gram-negative bacilli, they are rarely visualized on Gram stains of clinical material. A Gram stain of a sputum specimen showing polymorphonuclear leukocytes without bacteria can be a valuable clue to *Legionella* infection. *L. micdadei* is weakly acid fast when stained by use of the Kinyoun method or the modified Ziehl-Neelsen's method [92, 93], and it may, on occasion, be mistaken for a *Mycobacterium* species.

Isolation of *Legionella* species from a clinical specimen on selective media provides a definitive diagnosis. Buffered charcoal yeast extract agar that contains antibiotics to suppress commensal flora is commercially available [102]. However, certain media formulations that are selective for *L. pneumophila* may inhibit growth of other *Legionella* species. In particular, the addition of cefamandole to culture media will inhibit the growth of *Legionella* species that do not produce β -lactamase, such as *L. micdadei* and *L. bozemanii* [32]. A more sensitive media for isolation of non-*pneumophila* *Legionella* species is buffered charcoal yeast extract agar- α that contains vancomycin, anisomycin, and polymyxin B. Addition of the dyes

bromocresol purple and bromthymol blue to the media facilitates the identification of small colonies of *Legionella* species. A number of *Legionella* species show distinctive colony colorization on media that contain dye (figure 1). *L. micdadei* and *Legionella maceachernii* appear blue, and most other species appear pale green [103]. Use of dye also facilitates identification of infection by >1 *Legionella* species [84]. Acid pretreatment of sputum to inhibit commensal flora is an additional means of increasing the sensitivity of sputum culture. DFA testing of isolates provides confirmation of the identity of *Legionella* species.

Species-specific DFA antibody testing applied directly to clinical specimens offers rapid presumptive diagnosis of *Legionella* infection. It should be noted that the sensitivity and specificity of DFA testing of clinical specimens is not precisely known for species other than *L. pneumophila*. The commercially available *Legionella* urinary antigen test reliably detects only infection due to *L. pneumophila* serogroup 1 [104]. Urinary antigen test results are occasionally positive in cases of disease due to other *Legionella* species, but the sensitivity is low [36]; consequently, a negative test result is of little value in excluding *Legionella* infection. PCR detection of *Legionella* DNA in clinical specimens is a promising diagnostic technique [105]. The sensitivity and specificity of detecting seroconversion to *Legionella* species other than *L. pneumophila* is uncertain. While seroconversion alone can be used for the diagnosis of infection due to other

species, such diagnoses should be regarded as presumptive unless there are supporting microbiologic or epidemiologic data.

THERAPY

The vast majority of clinical experience in the treatment of *Legionella* infection involves *L. pneumophila*. However, other *Legionella* species share similar in vitro susceptibility patterns. Although clinical experience is limited, agents that are effective against *L. pneumophila* appear to be effective for the treatment of infection due to other species as well. *Legionella* species are susceptible to erythromycin, newer macrolides (azithromycin and clarithromycin), tetracycline, trimethoprim-sulfamethoxazole, fluoroquinolones, and rifampin [106–108].

Newer agents have replaced erythromycin, the historic agent of choice, as preferred therapy. Fluoroquinolones and the newer macrolides (clarithromycin, azithromycin, roxithromycin) are more active than erythromycin in assays of both in vitro and intracellular activity [106, 107]. In intracellular assays of drug susceptibility, quinolones are significantly more active against *L. micdadei* and *L. bozemanii* than against *L. pneumophila* [107].

Quinolones and newer macrolides have other clinical advantages over erythromycin, including once- or twice-daily dosing and excellent oral bioavailability. Macrolide antibiotics may have significant interactions with drugs used to suppress transplant rejection, including cyclosporine and tacrolimus. Fluor-

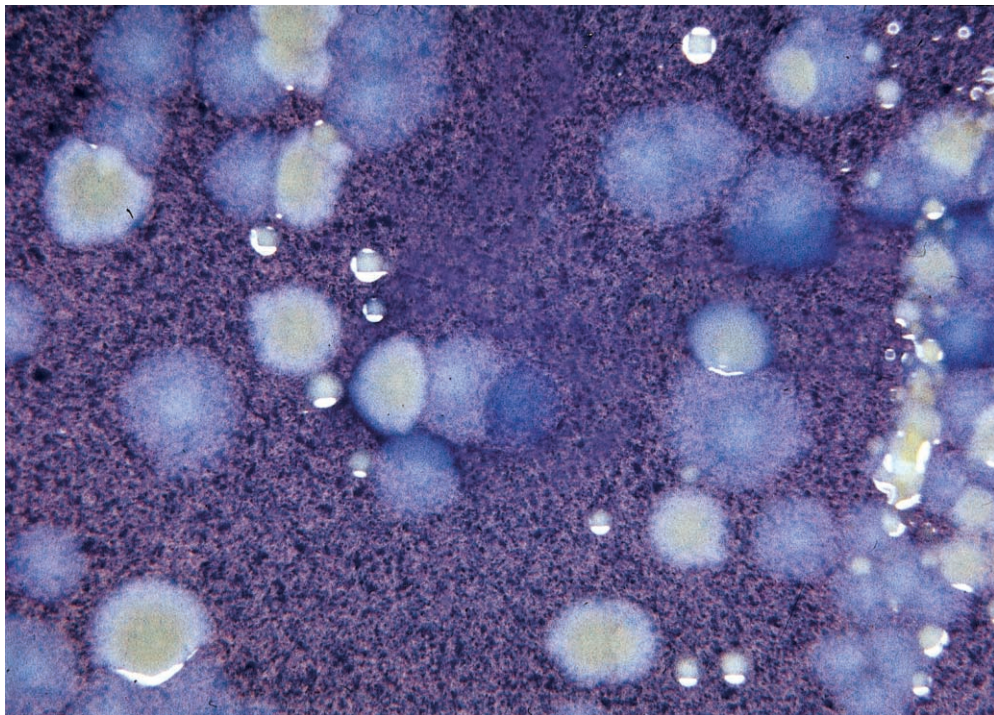


Figure 1. Growth of *Legionella* species on selective differential medium. *Legionella pneumophila* colonies appear light green; *Legionella micdadei* colonies appear blue.

quinolones are preferable for patients receiving these agents. Although adequate comparative trials are lacking, there are reports of failure of erythromycin in the treatment of *Legionella* infection in highly immunocompromised patients [109, 110]. Additional alternative regimens include doxycycline and the combination of rifampin and erythromycin, each of which have been used with clinical success after failure of erythromycin therapy [52].

PREVENTION

Many cases of pneumonia due to non-*pneumophila* *Legionella* species are sporadic; the source of infection is often unknown, but it is presumed to be an aquatic reservoir. As in the case of nosocomial disease due to *L. pneumophila*, clusters of nosocomial disease due to *L. micdadei*, *L. bozemanii*, and *L. dumoffii* have occurred in conjunction with isolation of these strains from hospital water supplies. Although there is considerable evidence that the presence of *L. pneumophila* is a hospital water system is predictive of the occurrence of nosocomial legionellosis within the facility [111–113], the risk posed by the presence of other *Legionella* species is less well defined. However, given the reports of clusters of nosocomial infection among susceptible patients, it would appear prudent to protect patients receiving high-dose corticosteroids, cytotoxic chemotherapy, or antirejection therapy from exposure to waterborne *L. pneumophila*, *L. micdadei*, *L. bozemanii*, or *L. dumoffii*. Measures directed against *L. pneumophila* will be effective in reducing levels of other *Legionella* species in potable water. These measures include shock chlorination [114] and superheating and flushing [40] (for emergency disinfection) and copper-silver ionization treatment [115] (as a long-term disinfection measure). Chlorination has been used as a long-term disinfection measure. It is no longer recommended; a major disadvantage is that maintenance of high chlorine levels may cause corrosion of pipes and leakage [116].

Other *Legionella* species may colonize hospital water as well. In one survey, 20% of hospital water systems were found to be colonized with *L. anisa* [117]; none of the participating facilities had diagnosed infections due to this species. Because none of these facilities routinely examined sputum samples for *Legionella* species, nosocomial infection may have been unrecognized. Until the risk posed by the presence of these less commonly encountered species is quantified, diagnostic evaluation of nosocomial pneumonia occurring in immunocompromised patients should include culture of respiratory specimens on media that will support the growth of multiple *Legionella* species.

In addition, culture on appropriate media has been critical in identifying non-respiratory tract infection, including soft-tissue infection, endocarditis, and nosocomial surgical site in-

fections due to *Legionella* species [45, 85, 100]. Clinical specimens from nonrespiratory sources are rarely submitted for culture for *Legionella* species; thus, the true spectrum of nonrespiratory *Legionella* infections is unknown. Inclusion of media selective for *Legionella* species in the laboratory evaluation of nonrespiratory infections of uncertain etiology may reveal a broader spectrum and higher incidence of disease due to *Legionella* species than has been previously recognized.

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