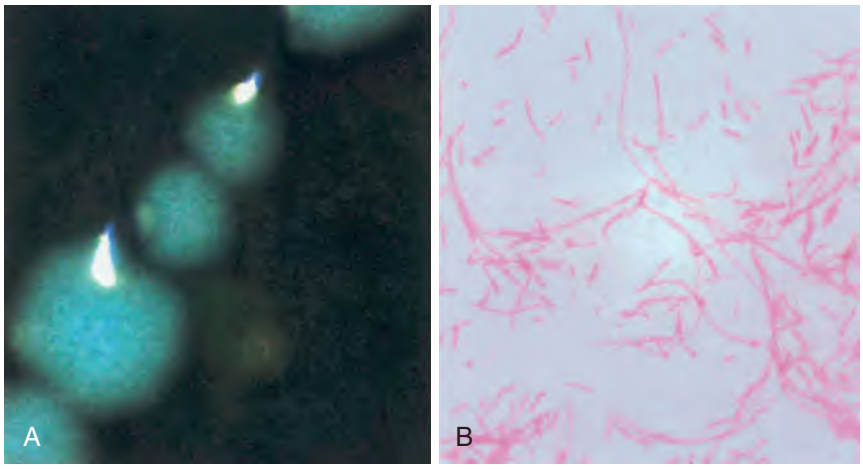

FIG. 232.1 Cultures of *Legionella pneumophila*. (A) Typical opal-like colony of *L. pneumophila* grown on BCYEα agar. (B) Gram stain of *L. pneumophila* taken from culture plate. Basic fuchsin should be used as the counter-stain as safranin stains the bacterium poorly.

A yeast extract agar containing iron, L-cysteine, α-ketoglutarate, and charcoal and buffered with an organic buffer (buffered charcoal-yeast extract [BCYEα] agar) is the preferred growth medium for clinical isolation. Clinically important *Legionella* spp. grow best at 35°C in humidified air on BCYEα medium, usually in 3 to 5 days after inoculation of plates. Up to 14 days' incubation may very rarely be required for the isolation of unusual *Legionella* spp.

More than 60 different *Legionella* spp. have been described, about half of which have been reported to infect humans.[29] *Lp* serogroup 1 (*Lp*1) caused the 1976 Philadelphia outbreak, and is the cause of 65% to 90% of all cases of LD for which there is a bacterial isolate.[30–32] *Lp*1 dominance is not universal. For example, *Lp*1 constituted only 66% of *Legionella* spp. isolates in LD patients in Ontario Province, Canada, with *Lp* serogroup 6 being a relatively common isolate in that province.[32] Also, *L. longbeachae (Llb)* causes 50% to 85% of LD cases in New Zealand, and a similar fraction in Australia.[33] *Lp*1 can be divided into multiple subtypes using a variety of serologic, other phenotypic, and genetic methods. One particular subtype of *Lp*1 causes 55% to 76% of cases of LD due to *Lp,* and 85% of cases due to *Lp*1. This "Pontiac" subtype reacts with a specific monoclonal antibody and contains the *lag-1* gene encoding a lipopolysaccharide acetyl transferase.[34,35] The most common non-*Lp* species that are isolated from humans are *Llb, L. micdadei, L. bozemanae,* and *L. dumoffii,*[31] which, with the exception of Australasia, constitute fewer than 5% of culture-proven cases.[36,37]

Most clinical microbiology laboratories should be able to identify *Legionella* bacteria to the genus level (Fig. 232.1). Identification of *Lp* and *Lp*1 can be accomplished by sophisticated laboratories. Identification of other *Lp* serogroups and other *Legionella* spp. is more difficult, requiring molecular testing for accurate identification.[38] Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry identification of bacterial colonies can be useful for the identification of common *Legionella* spp., but may not be completely accurate depending on the validity of the database, with custom databases increasing the accuracy of bacterial identification.

MICROBIAL ECOLOGY

The *Legionella* bacteria are found in our natural aqueous environment, in lakes, streams, and even coastal oceans, at temperatures ranging from 5°C to greater than 50°C.[39] Warm water (25°C–40°C) supports the highest concentration of these bacteria, with warm water being the major bacterial reservoir leading to LD. Free-living amebae in the same waters support the intracellular growth and survival of the *Legionella* bacteria[40,41] (Fig. 232.2). When faced with inimical environmental factors the *Legionella*-infected amebae encyst, allowing the survival of both the host and parasite. In both natural and man-made waters, *Legionella*-infected amebae are found in consortia of many different microorganisms, all of which exist in a biofilm.

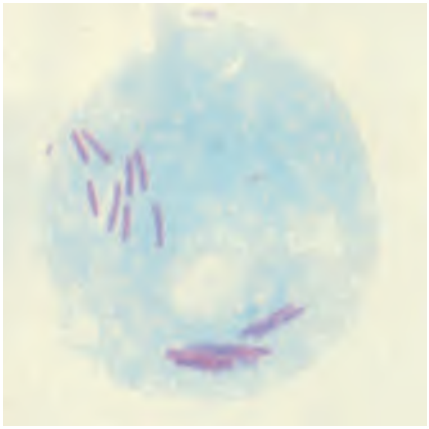

FIG. 232.2 Gimenez stain of *L. pneumophila* growing in an ameba. Note that the bacteria are much smaller and more uniform in morphology than when the bacteria are taken from a culture plate (see Fig. 232.1B).

In addition to intraamebal survival, free-living *Legionella* bacteria can enter a low metabolic state termed "viable but not cultivatable", making them difficult to recover from the environment and biocide-resistant.[42] The *Legionella* bacteria, amebae, and other microorganisms constantly escape from the biofilm because of pressure fluxes into a freely moving phase. Environmental changes that disrupt the biofilm can result in the sudden and massive release of *Legionella* bacteria into the surrounding water. If this water is then aerosolized or aspirated, the bacteria can cause illness in a susceptible host. Almost all cases of LD result from *Legionella* contamination of warm man-made water sources. *Legionella* is one of several opportunistic pathogens that are found in the built plumbing environment.[43] Some recently reported novel water sources include rain puddles in tropical regions, tsunami-related water exposure, windshield wiper fluid, water from truck tankers used to clean or maintain roads, and corroded domestic water pipes.[44–48] One exception to water as a risk factor is that *Llb* appears to be transmitted mainly through potting soil used by gardeners.[49] Water treatment of source water to make potable water may increase the concentration of *Legionella* bacteria (and mycobacteria) in the water.[50] *Legionella* bacteria are present in very low concentrations in disinfectant-treated cold potable water, usually at levels of less than 1 bacterium per liter. The bacterial density can be amplified by growth in biofilm within water distribution pipes, especially older pipes with low or no water flow. The bacteria can be further amplified in the presence of warm conditions, such as those found in many buildings or heat rejection devices. *Legionella* bacteria concentrations in air-conditioning cooling towers range from

10^2 to 10^8 colony-forming units (CFU)/L. Up to 80% of air-conditioning cooling towers tested contain the bacterium, as do 5% to 30% of home and industrial water heaters and hot water plumbing.[39] Contaminated water that is aerosolized serves as a disseminator of the bacteria into the environment. The concentration of *Legionella* bacteria in a particular environmental site may spontaneously fluctuate over a wide range.

PATHOGENESIS

Overview

LD is initiated by inhalation, and probably microaspiration, of *Legionella* bacteria into the lungs. Although *Legionella* bacteria are ubiquitous in our environment, they rarely cause disease. A confluence of a number of factors must occur simultaneously before LD is possible. These factors include the presence of virulent strains in an environmental site; a means for dissemination of the bacteria, such as by aerosolization; and proper environmental conditions allowing the survival and inhalation of an infectious dose of the bacteria by a susceptible host. Strains of different virulence exist for the same species, and some species and serogroups are more virulent than others.[51–53] Possible strain virulence factors include aerosol stability, ability to grow within macrophages, possession of eukaryotic gene homologs, and surface hydrophobicity.

The infectious form of the bacterium is not known, but in all cases the bacteria originate from water or soil. Several possibilities exist for the infectious particle, including bacteria contained within an amebal cyst, a sporelike form,[54] a biofilm particle containing *Legionella* bacteria and other bacteria, and freely dispersed extracellular *Legionella* bacteria. Virulence increases when the bacterium is grown in amebae, in the late stationary phase in vitro, or as the sporelike form.[55–57] The bacterial inoculum required to cause LD is unknown. Guinea pigs develop asymptomatic infection, disease, and death with inocula of 10 to 100, 1000, and 10,000 bacteria, respectively.[58,59] Bacteria in amebal cysts or in a biofilm fragment contain greater than 1000 bacteria, making it possible that inhalation of an infected amebal cyst or biofilm fragment could cause disease.[60] Survival of aerosolized extracellular *Lp* is dependent on relative humidity.[61] Relative humidity may be a key factor in disease transmission.[25,62] LD is a seasonal disease in some regions, with most cases occurring in the warmer months. For example, in the northeastern United States, the majority of cases occur from June through early October. This is probably because of elevated ambient temperature and humidity.[63]

After bacteria enter the lung, they are phagocytosed by alveolar macrophages. The bacteria produce virulence factors that enhance phagocytosis and promote intracellular survival and replication (see "*Legionella pneumophila* Virulence Factors" below). After sufficient intracellular replication, the bacteria kill the macrophage, escape into the extracellular environment, and are then rephagocytosed by macrophages. The bacterial concentration in the lung increases due to amplification of the bacteria within macrophages.

Following this intracellular multiplication, neutrophils, additional macrophages, and erythrocytes infiltrate the alveoli, and capillary leakage results in edema.[64] Chemokines and cytokines released by infected macrophages help trigger the severe inflammatory response. In the mouse model of LD, the relevant proinflammatory chemokines and cytokines include keratinocyte-derived chemokine (KC), macrophage inflammatory protein-2 (MIP-2), tumor necrosis factor-α (TNF-α), interleukin (IL)-12 and IL-18, and interferon-γ (IFN-γ).[65] Humans with LD had elevated levels of TNF-α and IL-8 in relation to other bacterial pneumonias in one study.[66] Systemic spread of the bacteria may be accomplished by infection of circulating monocytes.

The mechanisms for systemic toxicity of the disease are unclear, but it involves the severe inflammatory response to virulent *Lp.* Cytokine production is mediated by detection of microbial products by receptors of the innate immune system. Toll-like receptors (TLRs) on macrophages and other cells initiate host responses to both virulent *Lp* and avirulent mutants by detecting common pathogen-associated molecular patterns (PAMPs). TLR2 detects lipoproteins and lipopeptides, TLR5 flagellin, and TLR9 bacterial DNA.[65] Replication of *Lp* is greater in the lungs of mice deficient for TLR2 or the TLR adapter protein MyD88, resulting in higher mortality; and mice lacking TLR5 or TLR9 have delayed innate immune responses but disease susceptibility is not as pronounced as in TLR2-deficient mice.[65] Epidemiologic data indicate that humans with a TLR5 stop polymorphism are more susceptible to LD; for unclear reasons human TLR4 polymorphisms are protective[67–70] even though the lipid A moiety produced by *Lp* has an atypical structure and is less stimulatory toward TLR4 than the classic lipid A molecule produced by Enterobacteriaceae.[71]

The delivery of microbial products into the macrophage cytosol by virulent *Lp* contributes significantly to the robust inflammatory response. The mouse protein caspase 11 and the human homologues caspase 4 and caspase 5 bind directly to cytosolic lipopolysaccharide (LPS) during *Lp* infection of macrophages,[72–74] and caspase 11 has been shown to be essential for septic shock, implicating this pathway as being critical for LD pathology. Many responses are activated by host nucleotide-binding oligomerization domain (NOD)–like receptor (NLR) proteins. The mouse NLR protein NAIP5 detects bacterial flagellin to activate caspase-1–dependent processing and secretion of the cytokines IL-1b and IL-18, and animals that are defective for NAIP5 are significantly more susceptible to *Lp* infection. The NLR proteins NOD1 and NOD2 respond to peptidylglycan fragments delivered into the cytosol by virulent *Lp,* and sustain activation of innate immune signaling pathways. Lastly, the proteins RIG-I and MAVS respond to bacterial nucleic acid delivered into the cytosol during *Lp* infection to activate type I interferon production.[65]

Although the severe Th1 response induced by *Lp* can be detrimental to the host under high bacterial loads, these cytokines are crucial for the clearance of *Legionella* organisms.[75] Cytokines produced by infected alveolar macrophages are essential for the recruitment of neutrophils and for stimulating IFN-γ production by natural killer cells, which are both needed for sterilization in the lung.[76] Neutrophils are efficient at clearing extracellular *Lp* from the lungs. Macrophages activated by IFN-γ kill *Lp.*[77] This change in macrophage permissiveness involves, among other things, a reduction in intracellular iron, a factor that is necessary for *Lp* replication.[78] Indeed, the majority of legionellae seen in lung samples are associated with alveolar macrophages. Furthermore, the susceptibility of an animal species correlates with the ability of *Lp* to infect its macrophages, and bacterial mutants that are impaired for in vitro infection of macrophages have reduced virulence. Antibodies develop during the course of *Lp* infection, but the humoral immune response does not appear to be critical for host defense.

It is widely believed that the adaptation of *Lp* to protozoan niches in nature engendered it with the ability to infect mammalian phagocytes.[79] *Lp* enters the macrophage by conventional or coiling phagocytosis,[80–82] processes that utilize the host cell actin cytoskeleton.[83] Opsonization with the C3 component of complement can promote phagocytosis,[84] but entry by this pathway dampens the oxidative burst and thereby may enhance bacterial intracellular survival. However, opsonin-independent phagocytosis also appears to be important.[57] Even in the event that the oxidative burst is triggered, *Lp* strains may be resistant to hydrogen peroxide, superoxide anion, and hydroxyl radicals.

After entry, legionellae reside within a nascent phagosome (Fig. 232.3) that does not fuse with endosomes or lysosomes,[85–87] thereby avoiding acidification and degradative enzymes. The phagosome subverts host vesicles in the early secretory pathway using proteins produced by *Lp.*[88] The vacuole containing *Lp* rapidly recruits membrane from the endoplasmic reticulum and develops into an organelle that resembles the host rough endoplasmic reticulum.[89–91] This specialized *Legionella*-containing vacuole supports intracellular replication. This vacuole expands during replication and ultimately fills the host cell. Upon nutrient depletion (e.g., amino acid depletion), *Lp* enters stationary phase and converts to a flagellated form that is primed to seek out and infect new host cells.[55,92] Egress of *Lp* from the expended host cell is not well understood, but in macrophages this involves pathogen-induced apoptosis that leads to cellular necrosis.[93,94]

Neither the pathogenesis nor the etiology of PF is known with certainty. PF is caused by inhalation of a disease-causing environmental aerosol derived from water containing microorganisms including *Legionella* bacteria. Thirty percent to 85% of patients with this disease have serum anti-*Legionella* antibody in higher concentrations than is found in the normally healthy population.[95] The prevalent assumption is that the illness is caused by inhalation of the *Legionella* bacteria. Since

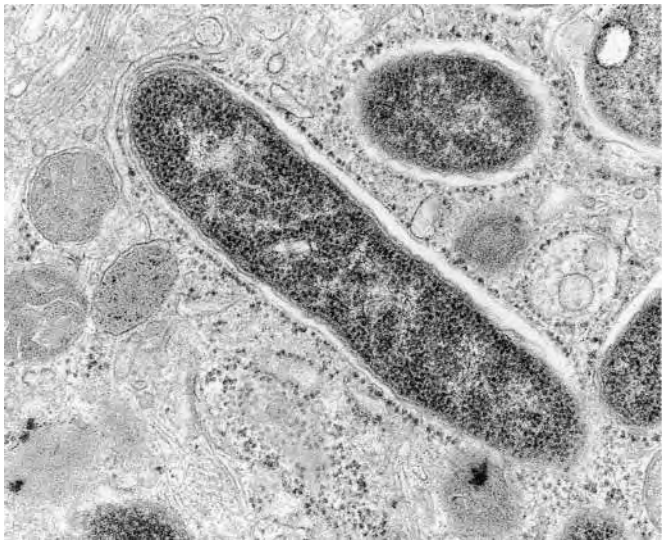

FIG. 232.3 Electron micrograph of *L. pneumophila* growing within a phagosome of an alveolar macrophage. Note the characteristic ribosomal studding of the phagosome.

the aerosols contain a mixture of microorganisms and endotoxins, it is unclear whether the disease is due to inhalation of endotoxin, of a polymicrobial aerosol, or of *Legionella* bacteria alone or to a combination of all these agents.[95] A study of the virulence for guinea pigs of the *Lp* strain that caused the 1976 Philadelphia LD epidemic and the PF environmental isolate from the Pontiac, Michigan, epidemic showed no differences between the two bacterial strains.[96] The incubation period of 4 to 6 hours of some PF patients supports a toxin-mediated illness, while the median incubation period of around 35 hours is more suggestive of initial bacterial multiplication causing illness. Infections with non-*Legionella* bacteria can produce *Legionella* antibodies, so the presence of such antibodies does not prove that PF is due solely to *Legionella* infection or intoxication. Bath water fever, a clinical syndrome thought to be due to endotoxin inhalation, is very similar to PF, suggesting that PF may also be caused by endotoxin inhalation.[95] Several reports exist in which exposure to the same environmental source led to PF in most exposed people, but to LD in a few people; whether the milder illness was really PF or mild LD is open to question, and does not help answer the question of etiology and pathogenesis.[95] Perhaps the strongest evidence implicating systemic infection with *Legionella* bacteria as the cause of PF has been the very rare reports of positive *Lp* urinary antigen tests or positive cultures in patients with PF.[95] The rarity of such cases and the rapid recovery without antibiotic therapy argue against systemic infection as the cause of PF.

Legionella pneumophila Virulence Factors

Studies of *Lp* grown in mammalian eukaryotic cells and in its natural environmental host, free-living amebae, show that the bacterium changes dramatically within cells as the intracellular infection progresses, becoming very small and highly motile as the cell loses its ability to sustain bacterial multiplication. These small motile forms, termed the "transmissive" type of bacteria, have increased virulence for eukaryotic cells. Once inside the cells, a nonmotile "replicative" phase predominates. The transmissive bacteria can be produced in vitro under nutrient-limiting conditions. Multiple bacterial factors control this transition between the transmissive and replicative phases, with some of the most important ones being production of ppGpp and activation of LetA/S and RpoS. CsrA plays an important role in the posttranscriptional regulation of this process.[97,98]

A variety of surface structures have been implicated in *Lp* pathogenesis. Type IV pili modestly promote bacterial attachment to macrophages and epithelial cells,[99] and flagella promote invasion independent of adherence.[100] The major outer membrane protein is a porin that also serves as a binding site for complement components and thus mediates opsonophagocytosis.[101] The Mip protein is a surface-exposed peptidyl prolyl isomerase that is required for the early stages of intracellular infection and for full virulence in animals.[102–104] Legionella LPS contains some endotoxic activity, and changes in LPS have correlated with increases in serum resistance, intracellular growth, and virulence.[105] Finally, the *rcp* gene, which appears to encode a lipid A–modifying enzyme, confers resistance to cationic peptides and promotes macrophage and lung infection.[106]

Lp secretes a variety of proteins, degradative enzymes, and putative toxins. The release of proteins by *Lp* into the extracellular milieu is mediated primarily by a type II secretion system.[107] Genome sequencing also suggests the existence of type I and type V secretion systems.[108] A number of enzymes and novel proteins are all secreted via the *Legionella* type II system.[107] Mutations within the genes encoding the type II secretion system diminish infectivity for macrophages, protozoa, and animals.[109,110] A secreted zinc protease is produced during infection and promotes pathology in the guinea pig model of disease as well as intracellular infection of some amebae hosts.[111,112]

A *Legionella* type IVb secretion system called Dot/Icm is essential for intracellular replication and virulence in animal models of disease.[113,114] It is essential for the ability of the *Legionella* parasite to modulate host vesicular transport to avoid delivery to lysosomes and promote vacuole biogenesis. Mutations in *dot/icm* loci lead to loss of virulence.[115,116] More than 300 proteins are secreted by the Dot/Icm system and in most cases these "effector" proteins translocate from the *Legionella*-containing vacuole into the host cell cytoplasm.[114] Some of these have been implicated in bacterial evasion of lysosome fusion.[117–119] Others, such as RalF, DrrA (SidM), LepB, LidA, and SidJ, play roles in the recruitment of endoplasmic reticulum–derived membranes to the *Legionella*-containing vacuole.[120–123] The effector SdhA is important for maintaining the integrity of the vacuole in which *Lp* resides,[124] and effectors SidF, SidP, and LepB directly modulate phosphatidylinositol phosphate signatures on the cytosolic surface of the vacuole that are important for the binding of other effectors.[125] *Lp* expresses several effectors that function as glucosyltransferases that are capable of inhibiting host cell protein synthesis (elongation factor 1A).[126,127] Although Dot/Icm is essential for *Lp* infection, a second type IV secretion system known as Lvh is found in addition to Dot/Icm in many strains; however, this system does not appear to deliver effector proteins into host cells but retains the ability to promote DNA conjugation, which could facilitate horizontal transfer of genes encoding effectors.[128] Interestingly, many of the type II and type IV secreted proteins as well as other mediators of infection (e.g., LpnE and Lpg0971) bear striking sequence similarity to eukaryotic proteins, suggesting that they were acquired by horizontal gene transfer from a eukaryotic host and use similar biochemical activities to facilitate *Lp* infection.[108,117,129–134]

Several infectivity factors have been localized to the *Lp* periplasm or cytoplasm. A Cu-Zn superoxide dismutase resides in the periplasm, affording resistance to toxic superoxide anions, and the KatB catalase-peroxidase is needed for optimal intracellular infection.[135,136] The *Legionella* phosphoenolpyruvate phosphotransferase and HtrA protein promote intracellular growth and virulence, and the phosphotransferase regulates expression of the transcriptional activator PmrA.[137–139] *Lp* iron acquisition, important for intra- and extracellular replication, involves, among other things, a secreted ferric iron chelator (the siderophore legiobactin), a secreted pyomelanin with ferric reductase activity, and an inner membrane ferrous iron transporter (FeoB).[140–143]

In addition to the identification of eukaryotic-like proteins in the *Lp* genome, another outcome of sequencing bacterial genomes is the realization that there are large segments of DNA, including plasmids and chromosomal "islands", that can vary between *Lp* strains.[108,144–146] It is possible that these variable regions of the genome will help explain differences in virulence that may exist between strains. Indeed, large deletions of the chromosome that remove individual islands can decrease *Lp* replication in specific protozoan hosts.[147]

Virulence Factors and Pathogenesis of Other *Legionella* Species

Relatively little is known about the virulence mechanisms, molecular pathogenesis, and cell biology of infections caused by *Legionella* spp. other than *Lp.* With the major exception of *Llb,* infections caused by

the other *Legionella* spp. are rare and found almost exclusively in severely immunocompromised patients. Genome sequencing of 38 different *Legionella* species revealed that there are over 5000 different effector proteins encoded by the genus and only seven Dot/Icm effector proteins that are shared by all species.[148] Thus effector plasticity, which facilitates adaptation of *Legionella* to different environmental hosts, likely influences human virulence potential. Despite unrestricted growth in otherwise nonpermissive macrophages, some non-*Lp* species fail to promote inflammatory cell death,[149] which could be correlated to a failure to cause disease in nonimmunocompromised people, although this is unstudied. *Llb* resides within a ribosome-studded phagosome, albeit with markers of late endosomal maturation, unlike *Lp,* which is able to block endosomal maturation at an early stage.[150–152] In contrast, *L. micdadei* resides in a smooth phagosome, and morphologic data suggest *L. dumoffii* grows within the cytoplasm rather than in a phagosome.[150,153–155] Analysis of the genome of several different *Llb* strains has shown that this species has and utilizes the Dot/Icm system for causing infection, that it is nonflagellated, and that it possesses a wide range of eukaryotic effectors different from those found in *Lp.*[156,157] Many of the *Llb* effectors appear to have been derived from plants and soil organisms, suggesting that it has evolved as primarily a soil-adapted bacterium. Experimental virulence of *Llb* for macrophages appears to be relatively independent of growth phase, in contrast to *Lp,*[151,158] and studies in mice and explanted human macrophages show that host antibacterial cytokines are suppressed.[157]

EPIDEMIOLOGY

Incubation Period and Contagiousness

The incubation period during most outbreaks of LD is between 2 and 10 days, with up to 10% of epidemic cases having incubation periods longer than 10 days. The median values are 4 to 6 days, with some outliers of from 1 up to 28 days.[1,159,160] A 2-month incubation period was reported for one nosocomial case.[161] The incubation period of PF is from 4 hours to 3 days, with a median of around 32 to 36 hours, although incubation periods of up to 5 days have been reported.[7,162,163] The incubation period of LD due to *Legionella* species other than *Lp* is not known with certainty; based on case reports from nosocomial cases of the disease, the incubation period after onset of immunosuppression appears to similar to that of LD due to *Lp.*[164]

Person-to-person transmission of LD or PF does not occur. A report of "probable" person-to-person transmission, the only such case ever reported, lacked sufficient scientific evidence to support this conclusion.[165] Laboratory transmission to humans has not been documented.

Patterns and Rates of Disease and Mortality

LD occurs in both sporadic and epidemic forms. For the years 2013–2014, about 3% of reported US cases were clustered, whereas in England and Wales from 2013 to 2015, about 20% of reported cases were clustered.[166–168] About 2% of reported cases are health care associated. In England and Wales for the same time periods, 40% of cases were acquired while traveling abroad. In the United States, about half the clustered cases were acquired by exposure to contaminated drinking water and the remainder by exposure to environmental water, such as from cooling towers. Underreporting of the disease is likely because of empirical treatment without laboratory testing, insensitive diagnostic tests, and the use of passive surveillance systems. Only 5166 cases of LD were reported in 2014 (16.2 per million population) to the Centers for Disease Control and Prevention (CDC). This rate is lower than that reported in Denmark, France, and Portugal in 2014, with 28.1, 20.5, and 56.4 cases per million population, respectively, and higher than that reported for England and Wales (5.8 per million).[36]

Prospective studies of both sporadic community-acquired and nosocomial LD have reported far more cases in the United States than would be expected based on the number of cases reported to the CDC. One study in Ohio of adults with community-acquired pneumonia requiring hospitalization found that 2.4% of such patients had LD, and that the disease incidence was approximately 80 per million population per year.[169] When extrapolated to the entire population of the United States, the authors estimated that between 8000 and 18,000 hospitalized cases of LD occur annually. The incidence of LD causing community-acquired pneumonia not requiring hospitalization is not known. One small regional US study estimated that the incidence of LD among outpatients treated for pneumonia was 40 to 280 per million population per year.[170] Thus somewhere between 18,000 and 88,000 cases of LD are estimated to occur per year in the United States, the majority of which are neither epidemic nor hospitalized. A German study of community-acquired LD found that the annual rate was 180 to 360 cases per million population, which if extrapolated to the United States would mean that 56,000 to 113,000 LD cases occur per year in the United States.[171] Some geographic regions appear to have more LD than others.[63] The incidence of LD in the United States and elsewhere appears to be increasing, based on the numbers of reported cases; whether this is due to more widespread use of the urine antigen test, better reporting and surveillance, or a true disease increase is unknown.[172] Estimates of LD as a cause of community-acquired pneumonia requiring hospitalization in adults range from 0.5% to 10% of all admitted pneumonia cases; an average value is probably approximately 2%, even in geographic regions with excellent diagnostic capabilities.[173–177]

LD in children is uncommon, representing 1% or less of causes of pneumonia, and occurs as a nosocomial disease of immunosuppressed children, and in neonates.[178] Nosocomial and domestically acquired cases of LD have been reported in apparently immunologically normal newborns exposed to *Legionella*-contaminated water in incubators and bath tubs, and during birth.[178–180]

Shortly after the 1976 Philadelphia outbreak, nosocomial LD outbreaks were reported in several cities throughout the United States and Europe. Because relatively little was known about the environmental ecology of *Lp,* or about optimal diagnostic methods, these outbreaks were characterized by long durations, often years in length, and high numbers of cases and fatalities. For example, the LD outbreak at the Wadsworth Veterans Administration Hospital in Los Angeles, California, resulted in more than 250 cases of disease in both patients and visitors over an 8-year period.[16,23,181] Nosocomial LD epidemics continue to occur worldwide, albeit with durations measured in weeks rather than years.[182,183] Nosocomial pneumonia usually affects a relatively small number of hospitalized patients, with attack rates less than 1% of patients.[16,184] During nosocomial outbreaks of LD, the minority (5%–11%) of patients with nosocomial pneumonia of all etiologies have been reported to have LD.[16,185–187]

Some recent large community-based LD outbreaks have been in New York City in 2015 (138 people, 16 deaths) and Flint, Michigan, in 2015 (91 people, 12 deaths). Several large outbreaks involved people visiting a town center, rather than being inside a certain building. Long-distance spread of disease from industrial aerosols has been reported.[25,188]

LD affecting travelers constitutes up to half of reported cases in some countries. In many cases, a common source outbreak has been found, but for others the cases appear to be sporadic. Multiple common source outbreaks have been uncovered in travelers by a cooperative European reporting system that collects and analyzes LD cases in Europeans.[189]

Sporadic cases of the disease are about 5-fold to 20-fold more common than linked cases of the disease. Some sporadic cases are undoubtedly the result of common source exposures. This is especially true of travelers who return to their homes during the incubation period, or while they are ill but still well enough to travel. Evidence for common source links for apparently unrelated cases comes from observations that proximity of residence to wet cooling towers is a risk factor for LD.[190,191] Residential acquisition from drinking and bathing water, which rarely results in more than a single case, accounts for less than 10% to 15% of sporadic LD.[192–194]

The reported incidences of LD in the United States rose almost fivefold from 2000 to 2016 (19 per million per year), and almost twofold from 2006 to 2016. The relative contributions of increased testing, aging of the population, increased immunosuppression of the population due to advances in medical care, and global warming are uncertain.

PF often causes explosive outbreaks of disease with high attack rates. Attack rates of 70% to 90% have been reported from several epidemics.[7,162,195–197]

LD mortality rates are variable, ranging from less than 1% to as high as 80%, depending on the underlying health of the patient, promptness of specific therapy, and whether the disease is sporadic, nosocomial, or part of a large outbreak.[30,198–201] The lowest mortality rates (approximately 1%), were observed in large outbreaks of the disease, whereas the highest mortality rates were reported in untreated nosocomial disease in patients with severe underlying diseases.[202] The average fatality rates for sporadic disease are estimated to be approximately 10% to 15%. Fatality rates of nosocomial disease have declined by more than 50% in the United States over the last 20 years; a similar but less dramatic decrease in death rates of community-acquired cases has also been observed.[30] The decline in mortality rates appears to be due to better and faster disease recognition and more widespread use of LD-effective empirical therapy for pneumonia.[30,202]

Risk Factors

Because of the ubiquity of *Legionella* spp. in our natural and man-made environments, most people encounter these bacteria frequently. Yet most of those exposed do not get the disease. This is attributed to largely unknown host immune mechanisms and to the lack of sufficient concentrations of virulent *Legionella* spp. bacteria in small aerosol particles. The known host risk factors for LD are those that result in decreased local or systemic cellular immunity, and those activities that increase the chances of exposure to an infectious aerosol or microaspiration of contaminated water. Also important are environmental and bacterial factors such as relative bacterial virulence, bacterial aerosol stability, organism growth conditions, and factors that facilitate the spread of the bacterium from contaminated water to the host, such as wind direction, relative humidity, and aerosol formation.

Male gender, cigarette smoking, chronic heart or lung disease, diabetes, end-stage renal failure, organ transplantation, immunosuppression, some forms of cancer, and age greater than 50 years are LD risk factors.[184,193,203,204] The approximately twofold greater risk for men may be due to the greater prevalence of cigarette smoking and its complications in males. As the population ages in developed countries, LD incidence will rise, based on the very high attack rates in the elderly, in the range of 800 to 1500 per million per year for those age 80 years and older.[32] Poverty, at least in large cities, is a risk factor for LD.[205] There may be racial disparities in LD incidence, with African Americans having a higher attack rate than whites, even after accounting for socioeconomic status.[205,206] Cigarette smoking and glucocorticoid administration both separately increase risk by approximately twofold to sevenfold. Anti-TNF-α therapy is a significant risk factor for LD, especially including TNF-α antibodies, but also thalidomide and lenalidomide.[207,208] Anti-CD52 therapy can result in severe LD.[209] Lung, but not gastrointestinal tract, cancer is an LD risk factor.[203] A variety of hematologic malignancies have also been shown to be important risk factors, especially hairy cell leukemia.[185,203] Small single studies of genetic predisposition to LD have shown that polymorphisms in TLR4, TLR5, and mannose-binding lectin produce minor predispositions to the disease.[67,69,210,211] Recent surgery has been an important risk factor for nosocomial disease.[212,213] Alcoholism is a predisposing condition in only some studies.[184,185,193,214] Host risk factors for non-*Lp* LD appear to be similar to those for *Lp* infection; most patients with these infections are immunosuppressed or have cancer, with the exception of LD due to *Llb*.[215,216] There appear to be no predisposing host factors for PF.

Activities that increase the chances of exposure to *Legionella* bacteria in water increase the risks of disease acquisition. Recent overnight travel, use of well water in the home, recent plumbing work inside the home, disruptions of water supply resulting in "brown" water at the water tap, and living in a water distribution network with older plumbing all increase the acquisition risk of community-acquired LD.[191,193,194,217,218] Increased risk activities include exposure to whirlpool or hot spring spas; living close to a cooling tower; and being near decorative fountains.[190,191,204,219,220] Rarely reported risks are near-drowning[10,44,220,221] and delivery by water birth.[222] Exposure risk factors for LD due to *Llb* are much different than for *Lp* infection, with handling of potting soil or compost, cigarette smoking, chronic obstructive lung disease, and not washing hands after use of the soil being major risk factors.[49]

A wide range of nosocomial exposures can result in LD. Almost all involve delivery of *Legionella*-contaminated water into the respiratory tract, and include the use of tap water filled or rinsed nebulizers, humidifiers, oxygen humidifiers, ventilator tubing, and nasogastric feedings or lavages.[223–226] Proximity to a contaminated decorative fountain has transmitted nosocomial LD in several outbreaks.[26,227] Consumption of *Legionella*-contaminated ice can also be a risk factor for nosocomial LD.[228] These are in addition to exposure to *Legionella*-contaminated air originating from a cooling tower.[229–231] Rare cases of nosocomial *Legionella* wound infection have resulted from irrigation or bathing of wounds in *Legionella*-contaminated water.[232,233]

Modes of Transmission

LD is transmitted from the environment to humans by inhalation of an infectious aerosol.[234] In an unknown fraction of cases microaspiration of contaminated water into the lungs is the mode of transmission.[226,235]

Multiple examples of exclusive aerosol transmission of LD exist, especially in epidemics having a cooling tower, water spa, water fountain, or water mister as the source of disease.[1,219,229,230,236–238] Drinking of water at the epidemic site has not been demonstrated to a be risk factor for LD.

The data supporting microaspiration of water as a major mode of transmission are less convincing. These data include examples of nosocomial disease in patients whose major risk factor was nasogastric tube irrigation with tap water, and interruption of nosocomial outbreaks by substituting sterile water for tap water for drinking and nasogastric tube irrigation.[226,235] Whether microaspiration is the major transmission mode for nosocomial disease is controversial and unproven.[239]

Rare reports of peritonitis or bowel abscesses caused by *Lp* have led to speculation that oral ingestion may be a mode of disease transmission.[240–242] In all these cases, *Lp* pneumonia or empyema occurred concurrently, making unclear which organs were the ones primarily infected. It is very unlikely that oral ingestion of *Legionella*-contaminated water is more than a very minor mode of transmission of the disease.

Outbreak Investigation

Prompt notification of public health authorities of any strongly suspected or confirmed case of LD is critically important for detecting epidemics of the disease, and is legally required in many regions. What appears to be only a single case of the disease may be part of an epidemic, or the index case.

Medical institutions should embark on extensive investigation of even a single case of nosocomial LD.[243] More cases may have occurred previously, or will appear subsequently. Retrospective review of nosocomial pneumonia cases for a 3- to 6-month period may yield more cases. Prospective laboratory testing of all patients with nosocomial pneumonia for LD for a 3- to 6-month period also can be useful. Hospital physicians should be notified to consider LD when making diagnostic and therapeutic decisions in patients with nosocomial pneumonia until it is clear that any ongoing nosocomial outbreak has been eradicated.

Investigation of both community-acquired and nosocomial outbreaks of LD requires a thorough epidemiologic investigation. Environmental testing without concurrent epidemiologic investigation can result in misleading findings, even when molecular fingerprinting is used to compare clinical and environmental isolates.[244–247] Not all environmental microbiology companies are competent in the proper collection, processing, and culturing of water specimens for *Legionella* spp. bacteria; certification is required in some countries, with a voluntary certification program available in the United States.[248] It is crucially important to obtain *Legionella* bacteria clinical isolates as part of an epidemiologic investigation; this allows molecular fingerprinting and comparison of clinical and environmental isolates. Environmental sampling protocols for outbreak investigation are available.[249,250]

Rough clues to the environmental source of an LD epidemic can be found in the pace of the outbreak and its geographic distribution. Explosive outbreaks involving tens to hundreds of people over a several-day period are most often due to a contaminated massive aerosol generator, usually a cooling tower but sometimes a whirlpool spa or misting device. Potable water–associated outbreaks may produce as many cases, but generally over a much longer period of time, such as many weeks

or months. Cooling tower–related epidemics often affect both building visitors and people who are within several hundred meters up to a 10-km distance from the tower.[25]

Environmental Decontamination for Outbreaks

All aerosol-generating sources implicated or highly suspected of being a source of epidemic LD should be taken out of operation as soon as possible. A number of guidelines exist for emergency disinfection of such sources, and these may differ according to local regulations or guidelines.[249–251] Long-term remediation can be complex and requires expert engineering and public health advice.

CLINICAL PRESENTATION

Legionnaires' Disease

LD causes acute consolidating pneumonia that cannot be accurately differentiated from pneumococcal pneumonia based on clinical or roentgenographic findings on initial presentation.[252–254] Initial clinical findings of both community-acquired and nosocomial epidemic LD in the 1970s seemed to show that a distinct clinical syndrome was observed.[255] This syndrome was characterized by fever with pulse-temperature dissociation, myalgia, nonproductive cough, few pulmonary symptoms, diarrhea, confusion, hyponatremia, hypophosphatemia, and elevated liver-associated enzymes. While this symptom complex does occur in LD, it is neither specific nor frequent enough to allow differentiation of LD from other common causes of community-acquired pneumonia. Clinical scoring systems to help increase diagnostic accuracy have not performed well enough to be used to guide proper therapy.[256–258]

The clinical findings of non-*Lp* LD do not differ significantly from those caused by *Lp*.[216,259] *Llb* LD occurs predominantly in nonimmunosuppressed patients, whereas the reverse is true for LD caused by the other non-*Lp Legionella* spp.[33] Those with immunosuppression and non-*Lp* LD tend to have a more severe course and greater likelihood of nonpulmonary LD, but this is similar to the findings of immunosuppressed patients with *Lp* LD.

A prodromal illness may occur, lasting for hours to days, with symptoms of headache, myalgia, asthenia, and anorexia. Fever accompanies this prodrome, except in severely immunocompromised patients and sometimes in the elderly. Multiple rigors may occur, as well as diarrhea and abdominal pain. Cough, with or without chest pain, may develop hours to days after onset of the prodrome; the cough produces purulent sputum in only approximately 50% of patients. The initial clinical picture may be confusing because the systemic symptoms can be more impressive than ones referable to the lower respiratory tract, leading some physicians to diagnose "influenza", a gastrointestinal illness, "sepsis", and in some cases an acute abdomen syndrome. These prodromal symptoms escalate in severity as the disease progresses, which may result in a several-day delay before presentation; the median time to presentation after onset of illness is approximately 4 days.[260] Careful physical examination of the chest, and chest roentgenography, almost always demonstrates findings of consolidating pneumonia.[261,262] Chest computed tomography findings include ground-glass opacities and consolidation.[263,264] Pleuritic chest pain, sometimes in concert with hemoptysis, can occur and may result in an incorrect diagnosis of pulmonary infarction. Headache may be the most prominent feature, and so severe as to suggest subarachnoid hemorrhage. Mental confusion is commonly reported; obtundation, seizures, and focal neurologic findings occur less frequently.[265–267] Some patients may have negative chest x-ray films on presentation but chest films taken a day later may show focal or diffuse pulmonary infiltrates; as with other causes of community-acquired pneumonia, plain chest films may be less sensitive than chest computed tomography examination at presentation.[268] Lung cavitation is seen almost exclusively in immunosuppressed patients.[269] Pleural effusion without pulmonary infiltrates is rarely observed. Bronchoscopic findings in LD patients are often remarkable for the absence of inflammation or purulent secretions in the large airways. Abdominal examination may reveal generalized or local tenderness and, in rare cases, evidence of peritonitis. Splenomegaly is uncommon. Findings of pericarditis, myocarditis, and focal abscesses are rare. No rash is associated with this disease, except that caused by other factors such as drug therapy. Symptoms of rhinorrhea, chronic afebrile fatigue, and fever without pneumonia lasting for many weeks are either not seen or so rare as to make the diagnosis unlikely.

Nonspecific laboratory test abnormalities may occur in LD[270] that are consistent with having a severe case of pneumonia. These include hyponatremia, hypophosphatemia, increased liver-associated enzymes, hyperbilirubinemia, leukopenia, thrombocytopenia, disseminated intravascular coagulation, leukocytosis, pyuria, elevated creatine kinase, and elevated lactate dehydrogenase. Patients with LD are more likely to have hyponatremia than do those with other causes of pneumonia, but the range of serum sodium values is too broad for this abnormality to be diagnostic in an individual patient.[271] Laboratory markers of pancreatitis are detected if this is a complication of LD. Renal disease caused by LD may result in the presence of urine casts and white blood cells, elevated serum creatinine levels, or both types of abnormalities. Myoglobinuria is a relatively common finding as indicated by a positive dipstick test for "blood" in the absence of significant numbers of red blood cells in the urine.

Clinical diagnosis may be more specific if the patient's clinical course after treatment has been given is taken into account, and if epidemiologic and immunologic risk factors are considered. The chances of a patient having LD are increased 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 intensive care unit hospitalization. Important epidemiologic clues include use of a hot tub or recreational spa; travel outside the home for one or more days; recent pneumonia of a coworker, fellow conference attendee, or fellow traveler; and recent plumbing work done at home or work.

The nonspecific presentation of LD can make clinical diagnosis very difficult, and mandates empirical therapy for this disease in most patients with community-acquired pneumonia of uncertain etiology. Diagnosis of the index or sporadic case of nosocomial LD is difficult because of the rarity of nosocomial LD in most hospitals, and because laboratory testing for nosocomial LD is uncommon in such settings.

Extrapulmonary Infections

Extrapulmonary infections are rare and usually occur as metastatic complications of pneumonia in immunocompromised patients. Metastatic infection has been reported almost exclusively in immunocompromised patients, or patients with fatal LD, who may develop abscesses and other infections of the brain, the spleen or extrathoracic lymph nodes, and skeletal and myocardial muscles.[272–276] Other reported sites of metastatic infection have been the intestines and liver, the kidneys, the peritoneum, the pericardium, vascular shunts and grafts, bone marrow, joints, surgical wounds (including those related to prosthetic heart valves and the aorta), native heart valves, the perirectal area, and the skin and subcutaneous tissues.[240,277–288] Both reported cases of culture-confirmed native valve endocarditis were in immunocompromised patients with LD.[288,289] In some of these cases the onset of symptoms of the metastatic infection preceded the recognition of pneumonia by several days, and in other cases the metastatic infection presented days to weeks subsequent to onset of the pneumonia. A metastatic infection site may be the only evidence of relapse of infection. Three cases of metastatic infection have been reported in apparently previously healthy patients,[274,290,291] but otherwise immunosuppression is found in such patients.

Very rare cases of primary infection not preceded or accompanied by pneumonia have been reported. Some of these appear to be the result of direct inoculation of *Legionella*-contaminated water into various tissues, usually in hosts with immune compromise. Some infections were introduced by bathing postoperative patients with contaminated tap water, by the use of therapeutic baths, and by inadvertent tap water irrigation of the mediastinum after esophageal perforation. Culture-proven sites of such infections have been surgical or other wounds, subcutaneous tissues, prosthetic and native joints, prosthetic heart valves, the mediastinum, and the respiratory sinuses.[232,233,286,291–303] In contrast to the case of native valve endocarditis, almost all patients with prosthetic valve endocarditis were neither immunosuppressed nor had LD.[292,293,296,297,304–306] Native and prosthetic joint infections have been reported uniformly in immunocompromised patients with preexisting arthritis, with one exception of a patient who was not immunocompromised but did have

underlying arthritis.[286,291,301–303,307] With one exception all joint infections occurred in the absence of LD. Polymerase chain reaction (PCR)–positive, but culture-negative, meningoencephalitis has been reported in patients without LD.[308]

Pontiac Fever

PF is a self-limited, short-duration febrile illness, usually diagnosed only during an outbreak of the disease.[7,95,162,163] Symptom onset is 4 to 60 hours after exposure to a bacteria-contaminated aerosol, either in a workplace or some other group setting. More than 80% to 90% of exposed people become ill. Unlike LD, children commonly get the disease.[95,162,163] The sources of contaminated aerosol have included industrial processes using sprayed water, recreational spas, decorative water fountains, and cooling towers. Fever, myalgia, headache, and asthenia are the dominant symptoms. Cough, dyspnea, anorexia, arthralgia, and abdominal pain occur less frequently. Most patients are not ill enough to seek medical attention, recovering without specific therapy 3 to 5 days after disease onset. There is little information about physical examination findings in the first day of illness; examination 2 to 5 days after onset may show fever and tachypnea. Pneumonia does not occur. Fatigue and nonfocal neurologic complaints may persist for up to several months in the minority of affected patients.[7] Because the clinical findings are nonspecific, it is very difficult, if not impossible, to accurately diagnose this disease in the absence of similar illnesses in coworkers or others with a common source exposure. Inquiries regarding the health of coworkers and acquaintances may help to confirm the diagnosis, but this can be nonspecific and insensitive.[309]

LABORATORY DIAGNOSIS

Specific, but relatively insensitive, tests are available for the diagnosis of LD (Table 232.1). Culture yield depends on the severity of illness, with the lowest yield (15%–25%) for mild pneumonia and the highest yield (>90%) for severe pneumonia causing respiratory failure.[38] Prior specific antimicrobial therapy affects yield adversely, although some patients have positive sputum cultures for days to weeks after initiation of specific therapy. Expectorated sputum or, even better, endotracheal aspirates are good specimens for culture; neither bronchoscopy nor lung biopsy is required for good culture yield, assuming a good-quality sputum specimen is obtained. Sputum Gram stain is insensitive. *Legionella micdadei,* and rarely *Lp,* may be acid fast or weakly acid fast.[310] Sputum cultures may be positive despite the presence of epithelial cells and lack of leukocytes, making suspicion of LD an exception to the usual sputum adequacy screening criteria. Blood culture is positive in approximately 10% of critically ill or severely immunocompromised LD patients, and in most patients with prosthetic valve endocarditis; specialized culture techniques are required to detect the bacteremia. Culture diagnosis is not dependent on *Lp* serotype or on *Legionella* spp., a fault of all of antibody and urine testing. Culture is also often the only test positive in cases of LD caused by other *Legionella* species. Culture media and selective conditions are optimized for the detection of *Lp,* with unknown performance for the detection of non-*Lp* species. Since a clinical isolate may be required for complete investigation of the source of an outbreak, culture for *Legionella* should always be performed regardless of the results of an antigen or PCR test. Many clinical laboratories have neither the expertise nor the ability to properly perform these specialized cultures.

Urine antigen testing is the most common laboratory test ordered for LD diagnosis.[30,311] This very specific test is easily performed by those without special skills and is often positive when other tests are negative. The test is not perfect because it is most sensitive for the detection of the Pontiac subtype of *Lp*1 (up to 90%), less sensitive for other monoclonal antibody types of *Lp*1 (60%), and very poorly sensitive (<5%) for other *Lp* serogroups and other species.[312] Since the majority (approximately 90%) of cases of community-acquired LD are due to the Pontiac subtype, the average sensitivity of this test is in the range of 70% to 80%. Immunocompromised patients, patients with nosocomial LD, and patients in some geographic regions are more likely to have LD caused by other serogroups and species, and hence a negative urine antigen test.[313] Yield can be increased by urine concentration, and for one assay type by prolonging the incubation time. Rare false-positive tests may be due to rheumatoid-like factors, easily inactivated by boiling; most laboratories do not use an inactivation step. A common clinical error is to order only urine antigen testing and to stop therapy for LD if the urine test is negative, because the urine test can be insensitive. Positive tests are associated with more severe disease.[214] Patients with extensive bilateral LD may excrete urinary antigen for weeks to months after recovery; this should not cause confusion over whether a positive test is the result of new or old pneumonia in the absence of a history of recent hospitalization for severe pneumonia.

Antibody detection is insensitive and of low specificity unless paired acute and convalescent sera are tested.[314] For optimal yield, convalescent sera should be collected at 4, 6, and 12 weeks after disease onset. Only approximately 75% of patients with culture-proven LD will seroconvert at all, even with optimally timed and tested sera. Commercially available serologic tests lack specificity or sensitivity because of deviations from the standardized test methods. Only seroconversion to *Lp*1 is of high enough specificity for clinical use; measurement of antibodies to other serogroups and species is plagued by low specificity and is not recommended.

Detection of *Lp* in respiratory tract tissues and fluids using immunofluorescent microscopy (direct fluorescent antibody [DFA] test) is very specific if a monoclonal antibody to this species is used and the test is performed by experts.[315] Use of other reagents, or testing by nonexperts, can result in many false-positive test results. The test is insensitive. Most laboratories do not perform this test because of its low yield and complexity. DFA staining can be useful for the detection of LD in fixed lung specimens.

Molecular amplification (PCR) and detection of *Lp* and non-*Lp* species is available at reference and public health laboratories.[38] No commercial PCR test approved by the US Food and Drug Administration is marketed in the United States. Most evaluations have shown that the molecular methods are about as sensitive as culture, with more recent studies showing that molecular methods are approximately 30% more sensitive than culture. The addition of PCR testing for *Lp* and *Legionella*

TABLE 232.1 Specific Diagnostic Tests for Legionnaires' Disease Caused by *Legionella pneumophila*

TEST	SPECIMEN TYPES	SENSITIVITY	SPECIFICITY	NOTES
Culture	Sputum, other lower respiratory tract secretions; lung; pleural fluid; blood; extrapulmonary tissues, fluids	20%–95%	100%	May be positive up to several days after treatment; requires special media and expertise
Antigenuria	Urine	60%–95%	>99%	Highest sensitivity for *L. pneumophila* serogroup 1, Pontiac type; may remain positive for days to months
Immunofluorescent microscopy	Same as culture	20%–50%	99%	Highest specificity with monoclonal antibody; requires very high level of technical expertise
Antibody	Paired serum	20%–70%	95%–99%	Highest specificity for *L. pneumophila* serogroup 1
Molecular amplification	Sputum, other lower respiratory tract secretions; urine	70%–95%	95%–99%	Excellent performance in some reference laboratories

[a]Pertains only to *L. pneumophila* infections. The yield of diagnostic tests is lower for infection caused by other species, especially those tests based on immunoassay.

spp. increases diagnostic yield by 10% to 100% over that for urine antigen testing, and appears to be most useful for the diagnosis of milder cases of the disease and for the detection of *Llb* infection.[316] A genus-specific PCR test, which can detect all known *Legionella* spp., can be quite useful for testing specimens from immunocompromised and other patients who may not be infected with *Lp.* Some studies have reported nonspecific PCR results; some reagents and nucleic acid extraction kits may be contaminated with *Legionella* DNA. Use of PCR for clinical purposes should only be undertaken for very well-validated and controlled assays, but when they are available these assays can increase diagnostic yield. Genetic sequencing of positive PCR products, or the use of whole-genome sequencing, in the absence of a positive culture, can be used in many cases for species-level identification and molecular fingerprinting.[317,318]

Optimal test yield requires performing more than one type of test; PCR (when available), sputum culture, and urine antigen testing are the preferred tests. If these are negative, and there are clinical or epidemiologic reasons for making a retrospective diagnosis weeks to months later, then antibody testing should be ordered. For patients who may be more likely to have LD caused by *Legionella* spp. other than *Lp*1, the best laboratory tests are sputum culture and PCR rather than urine antigen and serum antibody testing.

The yield of all tests except perhaps antibody determination is diminished by specific therapy, requiring testing before, or within a few days, of the start of antimicrobial therapy. Therapy should not be withheld pending collection or testing of specimens, and should not be stopped based solely on the result of a negative laboratory test.

THERAPY AND RESPONSE TO THERAPY

Legionella bacteria are intracellular parasites of monocytic phagocytes. This means that all antimicrobial agents efficacious for LD must be concentrated and bioactive in the correct subcellular location within these cells. The macrolides, quinolones, and tetracyclines all meet these criteria for *Lp* infection. Some of the non-*Lp* bacteria (e.g., *L. dumoffii, L. feeleii, L. jordanis*) are known not to reside in a phagosome,[154] making it possible that some of these drugs, which are differentially distributed within cells,[319] may be less effective than others for infections caused by these *Legionella* species. Tet(56), a tetracycline destructase that confers tetracycline resistance, has been reported to be present and active in one strain of *Llb*.[320] Proteins with sequence homology to Tet(56) are present in other *Llb* strains, *L. clemsonensis, L. massiliensis, L. nautarum, L. feeleii, L. quinlivanii,* and *L. geestiana;* apart from *Llb,* these other *Legionella* spp. either have not been reported to cause LD or are very rare causes of the disease. The presence of Tet(56) in *Llb* suggests that a tetracycline should not be used for the therapy of LD caused by this species, and that in regions where *Llb* infection is common tetracyclines are not drugs of choice for the empirical therapy of community-acquired pneumonia suspected to be LD. None of the β-lactams, monobactams, aminoglycosides, or phenicols is active for LD.[199]

Prospective adequately sized clinical trials of antimicrobial therapy for LD have not been performed.[199,257,321] Patients treated with erythromycin or a tetracycline drug had significantly lower fatality rates than did those treated with β-lactam or aminoglycoside agents in the 1976 Philadelphia epidemic.[1] In one outbreak of nosocomial disease, immunosuppressed patients treated with erythromycin had a 24% fatality rate, in comparison to an 80% rate for otherwise-treated patients, and erythromycin-treated nonimmunocompromised patients had a 7% fatality rate in comparison to 25% for those otherwise treated.[255] Small numbers of patients with LD have been reported to responded very well to erythromycin, a tetracycline, azithromycin, dirithromycin, clarithromycin, pefloxacin, ciprofloxacin, gatifloxacin, and levofloxacin. One retrospective study of LD with severe pneumonia showed that patients treated with a fluoroquinolone antimicrobial agent within 8 hours of ICU admission had significantly better outcomes than did those treated later, or with other drugs, including erythromycin.[322] Some more recent uncontrolled studies showed that erythromycin and clarithromycin were inferior to levofloxacin therapy for LD in terms of hospital length of stay, time to become afebrile, and complications, but not mortality[323,324]; of note, no comparison with azithromycin, the most potent anti-*Legionella* macrolide, was performed. Addition of rifampin to levofloxacin led to worse outcomes than levofloxacin alone in another uncontrolled study.[325] One study showed no significant difference in outcome for 104 patients treated with either levofloxacin, clarithromycin, or azithromycin.[326] Two recent large retrospective, propensity-adjusted studies showed that there is no significant difference in outcome for hospitalized LD patients treated with either azithromycin or levofloxacin, including in critically ill patients,[327,328] and a third large retrospective study of critically ill patients showed that levofloxacin therapy reduced mortality in comparison to non-azithromycin macrolides.[329] Combination antimicrobial therapy for LD is not beneficial, even in critically ill patients.[329] The available evidence supports the use of either azithromycin (500 mg daily for 3–7 days) or levofloxacin (500–750 mg daily for 5–10 days) for hospitalized patients with LD, with neither erythromycin nor clarithromycin being preferred therapies (Table 232.2).

Acquired resistance to antimicrobial drugs used to treat LD has not been detected in multiple in vitro susceptibility studies, including in unpublished studies of isolates from patients who had poor responses to therapy. *Lp* is known to develop drug tolerance when grown in macrophages,[330] and studies in guinea pigs show evidence of drug

TABLE 232.2 Preferred Therapy for Legionnaires' Disease

CLINICAL CONDITION	FIRST CHOICES	DOSAGE[a,b]	SECOND CHOICES	DOSAGE[a,b]
Mild pneumonia inpatient or outpatient, not immunocompromised	Azithromycin ***or*** Levofloxacin ***or*** Ciprofloxacin ***or*** Moxifloxacin ***or*** Clarithromycin	500 mg qd for 3–5 d 500 mg qd for 7–10 d 500 mg bid for 7–10 d 400 mg qd for 7–10 d 500 mg bid for 10–14 d	Doxycycline[c] ***or*** Erythromycin	200 mg loading dose, then 100 mg bid for 10–14 d 500 mg qid for 10–14 d
Moderate to severe pneumonia or immunocompromised[d]	Azithromycin ***or*** Levofloxacin ***or*** 750 mg qd for 5–7 d	500 mg qd for 5–7 d 500 mg qd for 7–10 d	Ciprofloxacin ***or*** Moxifloxacin ***or*** Erythromycin	400 mg IV tid initially, then 750 mg PO bid for 10–14 d 400 mg qd for 10–14 d 750–1000 mg IV qid for 3–7 d, then 500 mg PO qid for a total course of 21 d

[a]Dosage adjustments must be made for some of these drugs for patients with renal insufficiency. Patients with mild disease may be treated entirely with oral therapy, whereas for severely ill patients parenteral therapy is advised until improvement is seen and oral absorption is sufficient. Unless otherwise noted, all dosages given are for either IV or oral routes.
[b]Therapy duration may need to be considerably longer for patients with lung abscesses, empyema, endocarditis, or extrathoracic infection.
[c]Doxycycline should not be used to treat suspected or known *L. longbeachae* infection.
[d]Severely immunocompromised patients may require courses of therapy at the high end of the duration ranges given, or even longer. Close clinical follow-up is required to detect possible relapse after antibiotics have been discontinued.

tolerance for erythromycin and several other drugs.[331–333] Drug tolerance, possibly due to induction of efflux pumps,[334] was thought to be the cause of relapses in immunocompromised patients treated with erythromycin, and the overall poorer performance of erythromycin and clarithromycin therapy in critically ill patients. More recently, the preexistence and emergence of low-level quinolone heteroresistance during treatment for LD was reported, based on molecular studies during quinolone and other therapies.[335,336] There is also one report of the isolation of a clinical isolate with elevated ciprofloxacin and levofloxacin minimal inhibitory concentrations, but the relationship between these results and the response of the patient to ciprofloxacin therapy is uncertain,[337] with no apparent outcome difference between those patients with a heteroresistant population and those without one.[335] Since quinolone therapy is quite effective for the treatment of LD, and since dual quinolone-macrolide therapy does not improve outcome in critically ill patients, it is difficult to suggest combination therapy or nonquinolone therapy to counter the emergence of resistance to quinolone therapy.

The decision regarding which antimicrobial agent to administer for LD should be guided by severity of illness, degree of immunocompromise, drug cost, drug toxicity, and drug availability (see Table 232.2). Nonimmunocompromised outpatients with mild LD can be treated with any of the drugs listed in Table 232.2, with drug cost, availability, and toxicity being the main deciding factors. Hospitalized or immunocompromised patients with LD should be treated with one of the listed quinolones or azithromycin, unless drug unavailability or cost prevents their use. Initial intravenous antimicrobial therapy may be required in severely ill patients. Even in such patients, oral antimicrobial therapy can be used as soon as there is clinical improvement and intestinal drug absorption is adequate. Immunocompromised patients treated with erythromycin or clarithromycin, with or without rifampin, may suffer relapse days to months after cessation of therapy, especially if the level of immunosuppression is subsequently increased. In cases in which severe immunosuppression is chronic, thought should be given to continued suppressive therapy with a drug such as azithromycin, which has a very long intracellular half-life.[298]

The optimal duration of antibiotic therapy for LD has not been determined conclusively.[338] Use of short courses of azithromycin (500 mg/day), ranging from 3 days to 7 days in outpatients and inpatients, respectively, has been very effective, with only one relapse reported in a severely immunosuppressed patient.[339–341] Those with extensive pneumonia or with severe immunocompromise may profit by longer administration of azithromycin. Neither erythromycin nor clarithromycin are as active, or persistent in macrophages, as is azithromycin, requiring treatment durations of 14 to 21 (if immunosuppressed) days. Five days of treatment with levofloxacin (750 mg/day) has been shown to be very effective without relapses, and as effective as a 7- to 14-day course with 500 mg/day.[342] Overall, except for severely immunocompromised patients, the duration of antibiotic therapy should follow the general guidelines for all forms of community-acquired pneumonia.[343]

Rifampin should not be used to treat LD unless there are no other options available. Coadministration of rifampin with any of the drugs listed may be harmful, and has negligible benefit.[325] Rifampin dramatically reduces serum and lung concentrations of all of the macrolide drugs due to induction of drug clearance, with the enhanced clearance persisting for weeks after stopping the rifampin.[344–346]

Most LD patients treated with one of the recommended antimicrobial agents respond promptly to the therapy, sometimes within hours. Within 12 to 24 hours, most patients have improvement or complete clearance of myalgia, confusion, headache, abdominal pain, diarrhea, nausea, vomiting, and anorexia. Four to 7 days may be required for complete resolution of fever, with a steady decrease in fever over that period and with the most improvement being seen in the first day or two. Cough, sputum production, shortness of breath, and pleuritic chest pain respond more slowly to therapy, but major improvements usually occur within the first several days. As with most types of bacterial pneumonia, convalescence may be prolonged for months and complicated by neuropsychiatric disease, including chronic fatigue[347,348]; in addition there can be persistent (months) pulmonary physiologic abnormalities that may be symptomatic.[349] Evidence of chest consolidation on physical examination, and especially by roentgenography, takes considerably longer to resolve. It is common to observe apparent increases in the sizes of the original pulmonary infiltrates despite substantial clinical improvement over the first several days of therapy; this is not a cause for alarm as long as by other measures the patient is improving.[350] Complete clearing of the chest roentgenograph may not occur for up to 4 months after initiation of specific antimicrobial therapy, with the majority of patients having complete clearing by 2 months.[261,351,352] Severely immunocompromised patients, or those with severe pneumonia requiring artificial ventilation, may take longer to improve after initiation of specific therapy, or may not respond at all because of irreversible acute respiratory distress syndrome (ARDS); Even in such patients many of the systemic signs of infection (e.g., fever) improve, although the respiratory failure itself may worsen.

Failure to respond to specific therapy for LD should bring into question the validity of the diagnosis, the possibility of coinfection or superinfection, and the possibility of extrapulmonary disease complicating the LD. Up to 10% of patients with LD have coinfections or superinfections with other respiratory pathogens or other pathogens, such as *Pneumococcus, Haemophilus influenzae, Staphylococcus aureus, Enterobacteriaceae, Listeria, Nocardia, Pneumocystis, Aspergillus,* tuberculosis, *Cryptococcus,* and a variety of viruses.[257,353,354] Superinfection with opportunistic pathogens is generally seen in severely immunocompromised patients and in those in nosocomial LD, whereas coinfection with common respiratory pathogens may be seen in patients with community-acquired LD, based on positive blood or sputum cultures at the time of diagnosis of LD.[353–357] Drug fever, pancreatitis, myocarditis, hepatitis, pericarditis, metastatic infection, and pleural empyema may rarely be complications of LD, or its treatment, and causes of prolonged fever or poor response to therapy.

Ancillary therapy of LD has not been studied systematically. Correction of fluid and electrolyte abnormalities, and hypoxemia, is useful. Corticosteroid therapy for LD-caused ARDS is advocated by some,[257] but is of unproven benefit and could be detrimental to control of the infection.[260,358,359] A clinical response to administration of azithromycin or a quinolone is mandated before the administration of such immunosuppressive therapy, as well as continuation of the antimicrobials during and after steroid therapy. Corticosteroid therapy may be indicated for postpneumonic lung diseases such as cryptogenic organizing pneumonia, and perhaps pulmonary fibrosis.[360–362] Extracorporeal membrane oxygenation therapy can be lifesaving in patients with severe LD.[363]

PREVENTION

Immunization and Chemoprophylaxis

No human vaccine for LD exists, and prior infection does not necessarily prevent reinfection.[354] Antibiotic prophylaxis has prevented LD in immunocompromised patients during nosocomial epidemics of the disease.[364,365]

Engineering Modifications and Maintenance

Proper building, cooling tower, and plumbing design and construction can reduce the frequency and intensity of *Lp* contamination of potable water systems.[366] Risk assessment and a water management program are important parts of environmental control, and required by many governmental organizations.[367–369]

Use of monochloramine to treat public drinking water reduces *Legionella* spp. colonization of water, as well as rates of nosocomial LD.[370–372] Secondary disinfection with monochloramine also appears to be highly effective.[373,374]

Environmental Cultures for *Legionella* Bacteria

There is little national or international consensus regarding the benefit of routine environmental cultures to prevent LD. The *Legionella* bacteria present in our aqueous environment almost never cause disease. That, combined with natural fluctuations, imprecision in measuring bacterial concentrations, and extreme heterogeneity in *Legionella* environmental concentrations, make it difficult to define a specific and sensitive bacterial

concentration target for remediation. Many, but not all, government and other organizations recommend against the routine use of environmental cultures in risk assessment.[243,375–378]

Many bodies recommend that hospital water systems supplying wards housing immunocompromised patients be cultured for *Lp,* and that remediation be carried out if any positive cultures are found in these areas. Other organizations suggest that all hospital water supplies be checked.[377–380] Evidence from several LD nosocomial outbreaks shows that detection of any *Legionella* spp. in a hospital water distribution may be a cause for concern, and that a guideline previously used by some for remediation if 30% or more of cultured sites are positive is insensitive.[381,382]

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The complete reference list is available online at Expert Consult.

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233 *Capnocytophaga*

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SHORT VIEW SUMMARY

Definition
- Endogenous or zoonotic-associated infections have been reported, depending on the species of *Capnocytophaga* involved.
- Symptoms range from sepsis (most often) with and without central nervous system involvement to ocular disease; illnesses associated with pregnancy; and illnesses involving bones, joints, and soft tissues.

Epidemiology
- Endogenous infections arise in children or adults with blood dyscrasias and significant neutropenia.
- Sepsis arises from human oral species seeding into the circulatory system through abraded or damaged gums or gingival pockets in the oral cavity.
- Mortality rate is low (<3%) with appropriate treatment.
- Zoonotic infections arise primarily in male patients from a dog bite or close contact with dogs.
- Persons most at risk include dog owners, breeders, veterinarians, kennel workers, and hunters.
- Patients at highest risk for developing serious life-threatening infections with disseminated intravascular coagulation include patients who have undergone splenectomy or functionally asplenic patients and patients with ethanol abuse or immunosuppression.
- Reported mortality rates range from 13% to 33%.

Microbiology
- Bacilli are gram-negative with tapered ends.
- They are slow growing and fastidious, requiring 3 to 10 days of incubation on supportive media.
- No standardized method of determining drug susceptibilities exists, but testing should be attempted, if possible.

Diagnosis
- Is history compatible with *Capnocytophaga* infection (dog bite)?
- Does Gram or Wright-Giemsa stain of blood or other sterile specimens show bacilli with tapered ends sometimes within polymorphonuclear leukocytes?
- Conventional tests that include catalase, oxidase, and arginine dihydrolase are slow but reliable.
- Rapid molecular tests include 16S ribosomal RNA gene sequencing for rapid diagnosis on specimens or isolated organisms, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for identification of isolated organisms, and whole-genome sequencing.

Therapy
- Carbapenems and penicillin/β-lactamase inhibitor combinations are the first-choice drugs in serious infections caused by human oral *Capnocytophaga*. Resistance to a number of drug classes including β-lactams is frequently observed owing to β-lactamase production by human oral *Capnocytophaga*.
- A broader range of treatments is available for zoonotic infections. *Capnocytophaga canimorsus* recovered from dogs is not known to produce β-lactamases.
- Acquired drug resistance has been reported in some isolates.

Prevention
- Clinicians must be made aware of these organisms, the diseases they cause, and the patients at risk.
- Laboratories may require extra incubation time, a specialized incubation atmosphere, and richer media to isolate these organisms; clinicians should inform laboratory personnel about clinical suspicion so that appropriate culture methods are used.
- Rapid testing methods should be used to decrease time to diagnosis.

The genus *Capnocytophaga* consists of nine or more gram-negative fastidious species that typically reside as normal flora in the oral cavities of humans and other vertebrates including dogs and cats. Members of this genus can cause serious life-threatening infections in humans including septicemia and meningitis as a direct consequence of inapparent injuries or penetrating trauma resulting from animal contact or bites, most often involving dogs. Persons with underlying conditions including asplenia, alcoholism, and immunosuppression (neutropenia) are at greater risk of developing serious and fulminant systemic disease due to *Capnocytophaga*. The primary pathogen of this genus is *Capnocytophaga canimorsus,* formerly known as dysgonic fermenter 2 (DF-2). The number of *Capnocytophaga* infections may be increasing in the general population due to many factors including increasing pet ownership, animal-associated occupations, and a greater number of immunocompromised persons.

TAXONOMY

The genus *Capnocytophaga* consists of fermentative capnophilic bacteria that morphologically appear as fusiform thin to slender gram-negative bacilli with tapered ends. The genus currently consists of nine species—*C. canimorsus, C. canis, C. cynodegmi, C. gingivalis, C. granulosa, C. haemolytica, C. leadbetteri, C. ochracea, C. sputigena*—that can be subdivided into two major groups associated with the oral microbiota of humans and animals.[1,2] A tenth and eleventh reputed species, *C. stomatis* and *C. endodontalis,* have been recovered from animals and human infections[1] and a periapical abscess,[3] respectively. All validated species or taxa are recognized human pathogens or implicated in causing disease.[1,2] Phylogenetically, the genus *Capnocytophaga* resides in the family Flavobacteriaceae. The complete genomes of six different *Capnocytophaga* spp. have been sequenced more recently.[4]

PATHOGENESIS AND CLINICAL MANIFESTATIONS

Few studies have focused on which virulence factors are important in oral-associated *Capnocytophaga* infections. Oral-associated *Capnocytophaga* organisms produce a number of cell-bound or extracellular factors that may promote progression of periodontitis by enhancing the growth of bacteria in plaques and subgingival pockets or by evading host immune responses. Such factors include immunoglobulin A1 (IgA1) protease, phospholipase A_2, aminopeptidases, and type IX secretion system as well as the ability to form biofilms and chemotaxis-guided (gliding) motility.[5,6]

Progress has been made in understanding potential pathogenicity mechanisms functional in zoonosis-associated invasive infections typified by fulminant *C. canimorsus* septicemia.[7] These mechanisms can be broadly categorized into four areas: (1) failure of *C. canimorsus* to trigger

innate immune responses based on resistance to complement-mediated lysis and evasion of phagocytosis by macrophages; (2) inability of Toll-like receptor 4 to respond to *C. canimorsus* with activation of proinflammatory responses including generation of tumor necrosis factor, cytokines (interleukin-6), chemokines (interleukin-8), and nitric oxide[8]; (3) expression of cell surface lipoproteins (polysaccharide utilization loci) and sialidase, which deglycosylate host glycoproteins (including human IgG), leading to bacterial growth and persistence in vivo; and (4) a modified lipid A and lipopolysaccharide architecture that may affect endotoxicity and binding to human myeloid differentiation factor 2.[9,10]

More recent studies indicate that only a subset of *C. canimorsus* strains may be highly pathogenic for humans.[11] Of nine known capsular serotypes of *C. canimorsus,* serotypes A, B, and C predominate, causing almost 90% of human infections. In contrast, these serotypes are found at a much lower frequency (7.6%) in the oral microbiota of dogs.[12] This fact may help account for the relatively low frequency of *C. canimorsus* infections in people after dog bites.

Human *Capnocytophaga* infections can be broken down into five major categories: (1) septicemia, (2) diseases of the central nervous system (CNS), (3) eye infections, (4) illnesses associated with pregnancy, and (5) miscellaneous complications including infections of bone and tissue. Although most *Capnocytophaga* infections occur in individuals with impaired immune function or with significant underlying disease, infections in healthy individuals have been well documented.[13–15]

Human Oral-Associated Species

Oral *Capnocytophaga* spp. are residents of the subgingival sulcus and of supragingival plaque and have been implicated as opportunistic pathogens involved in gingivitis, periodontal disease, and oropharyngeal mucositis.[16] Carriage rates of these fastidious bacteria in the oral cavity have been reported as high as 100% with species distribution and prevalence varying from study to study.[16] One study using two different molecular approaches found *C. ochracea* and *C. granulosa* to be the two most common species associated with subgingival plaque.[17] These results closely mirror findings from a multicenter study in which *C. ochracea* predominated as the cause of *Capnocytophaga* bacteremia in patients with cancer.[18] A 2016 review of all oral-associated *Capnocytophaga* infections reported between 2000 and 2016 found *C. ochracea* as the etiologic agent in 59% of cases and *C. sputigena* as the etiologic agent in 29%, with the remaining illnesses (12%) divided among several species.[16]

The most common serious infection associated with oral *Capnocytophaga* infection is septicemia. Sepsis is most often observed in patients with underlying hematologic malignancies including acute and chronic myelogenous leukemia, non-Hodgkin lymphomas, Hodgkin disease, and multiple myeloma (Table 233.1).[18–20] Onset of sepsis typically coincides with the initiation of profound neutropenia (<500 granulocytes/mm^3) induced by chemotherapy or after hematopoietic stem cell transplantation.[21] Oral ulcerations such as severe mucositis, gingival hyperplasia, esophagitis, and periodontitis appear to serve as portals of entry for systemic invasion. Maury and associates[19] found all 24 patients with *Capnocytophaga* bacteremia in their study to be neutropenic; 88% of these patients had severe oral mucositis or periodontitis. Warren and Allen[22] suggested that pediatric patients are more prone to developing *Capnocytophaga* sepsis than adults; Campbell and Edwards[23] reviewed the literature on pediatric infections caused by *Capnocytophaga* spp. and found bacteremia as a common feature of infection in all 16 immunocompromised children. In contrast, Jolivet-Gougeon and coworkers[24] found that the frequency of oral carriage of *Capnocytophaga* organisms by children hospitalized on an oncology service ranged from 16% to 61%, and no cases of systemic disease attributed to *Capnocytophaga* were detected during this 10-year period. These latter results suggest that although oral carriage of *Capnocytophaga* organisms by children may be high, the risk for developing severe disease is relatively low.

Bloodborne disease caused by human oral *Capnocytophaga* spp. is typically monomicrobial (85%–90%). When polymicrobial infections occur, they most often involve viridans streptococci, anaerobes, or aerobic or facultatively anaerobic rods that represent other oral microbiota. *C. ochracea* and *C. sputigena* are the most commonly implicated species in sepsis, with most cases occurring in immunocompromised persons.[16,18,19] However, serious *C. ochracea* sepsis and purpura fulminans were reported in a healthy 46-year-old man 2 weeks after an uneventful dental extraction.[25] Rare cases of *C. gingivalis* or *C. haemolytica* bacteremia have been reported.[16] The overall attributable mortality rate associated with *Capnocytophaga* bacteremia ranges from 16% to 42%; recent investigations have reported lower values, approaching 0%.[18,19,22,23] Fatal cases of *Capnocytophaga* bacteremia caused by oral-associated species continue to be described on occasion.[20]

TABLE 233.1 Salient Features Distinguishing Human-Associated From Animal-Associated *Capnocytophaga*

CHARACTERISTIC	HUMAN-ASSOCIATED	ANIMAL-ASSOCIATED
Patient Population		
Children	+	–
Adults	+	+
Underlying Diseases[a]		
Leukemia, lymphoma	+	–
Asplenia	–	+
Ethanol abuse	–	+
Risk Factors		
Neutropenia and chemotherapy	+	–
Dental manipulations	+	–
Animal bites	–	+
Laboratory Tests		
Catalase	–	+
Oxidase	–	+
Arginine dihydrolase	–	+

[a]Predisposing for invasive disease, such as septicemia.

Oral species are less frequently implicated in CNS infections than their zoonotic counterparts. In contrast to septicemia, CNS illnesses most often manifest in immunocompetent individuals, with the major risk factor being dental manipulations (see Table 233.1). Cases of *Capnocytophaga* subdural empyema and frontal brain abscess, sinusitis, and pleuropneumonitis have been described in healthy individuals who underwent various dental procedures including tooth extraction and orthodontia.[16] Brain abscesses and extremely rare cases of meningitis also have been reported in pediatric patients with blood dyscrasias.

Ocular infections can run the gamut from blepharoconjunctivitis to keratitis, endophthalmitis, and corneal ulceration. People prone to developing ocular disease are older than 70 years of age, immunosuppressed, or involved in intravenous or crack cocaine drug use. In one series of 10 patients with keratitis, risk factors associated with infection included corneal epithelial defects, previous ocular infections, topical corticosteroid therapy, and intraocular surgery.

Chorioamnionitis is the most common clinical presentation associated with pregnancy and *Capnocytophaga* infection. Mild-to-severe illnesses can develop in women, resulting in premature contractions, labor, or fetal death. *Capnocytophaga* spp. can be recovered from infected placenta, meconium, cervix, and endometrium as well as amniotic fluid. Perinatal illnesses may ensue via an ascending route of infection or through hematogenous spread.[16,26] Many cases of chorioamnionitis involve recent oral sex as the primary risk factor with a report of *Capnocytophaga* spp. recovered from dental plaque of a patient's partner.[27] Contemporary studies suggest the possibility of an increasing incidence of *C. sputigena* bacteremia in neonates or infants. Such perinatal infections are typically found in low-birth-weight (790–1820 g) preterm infants with gestational ages ranging from 22 to 29 weeks.[27,28] Maternal findings in reported cases are consistent with chorioamnionitis. The neonatal mortality rate reported for 15 cases was 6.7% in one study.[27]

Unusual monomicrobial or mixed infections involving oral *Capnocytophaga* spp. include peritonitis, pyogenic arthritis, vertebral osteomyelitis, cervical and liver abscesses, aspiration pneumonia, pleuropneumonitis, pleural effusion and empyema, pyonephrosis, and soft tissue infections such as pyomyositis of the iliopsoas muscle.[16]

Infections Associated With Zoonotic Species

The zoonotic species *C. canimorsus* and *C. cynodegmi* are normal inhabitants of the oral cavity of dogs and cats.[29,30] Between 58% and 70% of dogs harbor *C. canimorsus* in their oral cavity,[11,31] whereas comparable numbers in cats vary from 15% to 57%.[7] In many case reports, predisposing risk factors and apparent routes of infection are not identified, although Dilegge and associates[29] found that several *Capnocytophaga* spp. could be recovered from the mouths of dogs.

Most life-threatening illnesses associated with these two zoonoses are attributed to *C. canimorsus* (>90%), which seems to have a higher predilection for causing serious disease than *C. cynodegmi*. Zoonotic-associated infections (septicemia, meningitis) arise via exogenous introduction of bacteria into wounds from penetrating trauma (dog bite) or by unapparent inoculation of bacilli into surfaces or tissues via close contact with pets. Such complications can result from innocuous activities associated with pet ownership including close petting, intimate contact (kissing), minor scratches, or the licking of minor abrasions or apparently intact skin. Invasive diseases resulting from such exposures are associated with higher mortality rates than endogenously acquired infections.

Symptoms associated with *C. canimorsus* septicemia are nonspecific and similar to symptoms of other gram-negative pathogens including fever, diarrhea, abdominal pain, vomiting, headache, and confusion.[32] A prominent diagnostic feature, found in 20% to 40% of cases of *C. canimorsus* septicemia, is a rash on the trunk or extremities or both that may vary from a macular or maculopapular eruption to a more severe and rapidly fatal form such as purpura fulminans with petechial lesions, retiform purpura, urticarial lesions, or symmetrical gangrene.[33–35] The overall case-fatality rate for *C. canimorsus* septicemia varies from 13% to 33%,[36,37] but mortality rates can approach 60% to 80% in patients with septic shock and multiple organ failure.[11,38] A review of more than 400 cases of *C. canimorsus* bacteremia estimated the overall case-fatality rate to be 26%.[7]

Meningitis is the second most common presentation, and the reported mortality rate is very low (3%) compared with cases of septicemia without CNS involvement.[7,39] At least 96% of patients with meningitis present with two of four symptoms including headache, fever, neck stiffness, and altered mental status.[39] *C. canimorsus* has also been linked to endocarditis, with a fatality rate approaching 25%.[40] Other miscellaneous infections have been described including brain abscess, sacral epidural abscess, eye and bone or joint infections, cellulitis, glomerulonephritis, renal failure, and pneumonia.[7,33,41,42]

EPIDEMIOLOGY

Several studies have estimated the annual incidence of *C. canimorsus* infections at 0.5 to 1.0 infection/1 million population per year,[36–38] or about 150 to 300 cases in the United States each year. *C. canimorsus* sepsis most commonly occurs in men (male-to-female ratio 2.7–3.8:1) older than 50 years of age (70%–90%) with one or more underlying conditions (62%–89%).[33,43] Past medical history often reveals recent dog bite exposure or incidental contact with dogs (60%–84%),[7,37,44–46] with the onset of symptoms occurring 1 to 8 days after exposure.[7,45] People at risk for developing bloodborne disease include dog owners, veterinarians, breeders, kennel workers, mail carriers, and hunters. On at least six occasions, cat bites or scratches have been implicated as the source of *C. canimorsus* or related species septicemia, with one reported fatality.[2,47] More than 400 cases of systemic infections involving *C. canimorsus* have been published in the literature, with one retrospective study reporting on 55 cases of invasive disease over a 32-year period.[7,32] Some surveys suggest that detection of systemic disease caused by *C. canimorsus* may be increasing because of several factors including increasing pet ownership, more opportunities for animal contact, a higher number of immunocompromised persons in the general population, and better molecular methods in the laboratory for detecting infections.

Patients who have undergone splenectomy or functionally asplenic patients are more prone to developing sepsis with disseminated intravascular coagulation than individuals with intact spleens, and multiple case reports describing overwhelming postsplenectomy infections accompanying *C. canimorsus* sepsis have been reported.[48] The incidence of patients with *C. canimorsus* bacteremia and asplenia is 13% to 33%.[7,33,37] Other underlying conditions associated with aggressive *C. canimorsus* infections include ethanol abuse (20%–31%), immunosuppression (3%–6%), rituximab, and corticosteroid therapy.[7,33,37,49] Job and associates[50] estimated that patients with underlying medical problems are three times more likely to contract *Capnocytophaga* disease than healthy individuals, although fatality rates are not appreciably different. In addition to disseminated intravascular coagulation, other complications associated with *C. canimorsus* septicemia include Waterhouse-Friderichsen syndrome, Stevens-Johnson syndrome, thrombotic thrombocytopenic purpura, and hemolytic-uremic syndrome.[51–53] In 30% to 40% of infections, no recognizable predisposing factor is evident,[38] and up to 40% of serious life-threatening *C. canimorsus* illnesses can occur in apparently healthy people.[43,54]

Although *C. cynodegmi* is a common resident of the mouths of dogs and cats, it rarely causes disease.[46] Differences in pathogenicity between *C. canimorsus* and *C. cynodegmi* appear to be unrelated to complement-mediated lysis, as both species are readily killed by human serum.[55] A fatal case of *C. cynodegmi* sepsis and meningitis in a 72-year-old woman who had previously undergone splenectomy and who was bitten on the hand by her pet dog has been described.[56] She rapidly developed signs of sepsis, with facial purpura and a progressive macular rash, and died of infection within 48 hours of admission. *C. cynodegmi* peritonitis has been reported in a 67-year-old man[57] and in a 39-year-old woman on continuous or intermittent peritoneal dialysis.[58] In both cases a pet cat was suspected as the source of infection.

DIAGNOSIS AND LABORATORY IDENTIFICATION

Presumptive diagnosis of *Capnocytophaga* sepsis can be made by examination of the patient's peripheral whole blood or buffy coat using Gram or Wright-Giemsa stain.[59] The presence of *Capnocytophaga* should be strongly suspected if slender, medium to long, gram-negative rods with tapered ends are observed (Fig. 233.1). Pers and colleagues[60] reported seeing bacteria on initial microscopic examinations of cerebrospinal fluid (CSF), whereas another report described slender, fusiform bacilli in conjunctival scrapings.[61] 16S ribosomal RNA (rRNA) gene sequencing performed directly on a clinical sample or a positive blood culture or CSF can decrease the turnaround time to diagnosis,[59,62] and polymerase chain reaction (PCR) assay using species-specific primers can detect *Capnocytophaga* in mixed culture in dental plaque samples.[63]

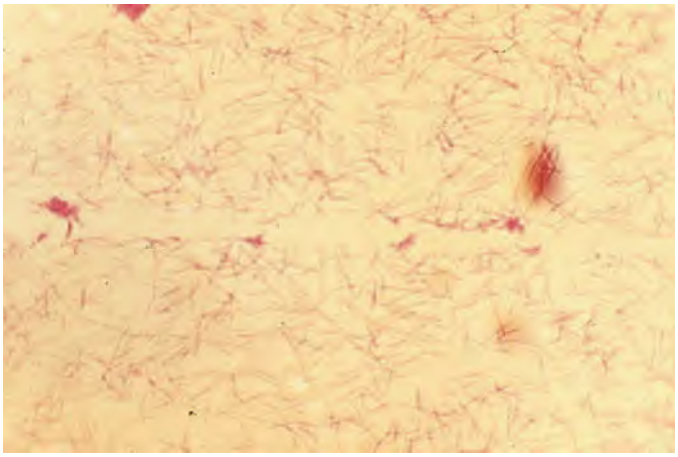

FIG. 233.1 Gram stain of *Capnocytophaga* exhibiting long, slender rods. *(Courtesy Dr. Edward J. Bottone.)*

Despite the widespread application of molecular techniques to identify organisms from clinical samples, most clinically significant isolates of *Capnocytophaga* are detected by culture. Clinical suspicion of *Capnocytophaga* septicemia should prompt a request to the laboratory to incubate blood cultures for up to 10 days or longer to enhance recovery of this fastidious, often slow-growing organism.[64] Recovery of capnocytophagae from CSF or blood typically takes 3 to 7 days. Some *Capnocytophaga* spp. appear to be sensitive to the anticoagulant sodium polyanethole sulfonate used in some blood culture bottles. One report cited recovery of *C. canimorsus* after 25 days of incubation.[40] Performing a Gram stain on blood culture sediment at the first indication of growth can yield a presumptive identification, although there is a small chance that the Gram stain can be misread.[65] *Capnocytophaga* spp. can be recovered from other clinical specimens including CSF, abscess material, respiratory secretions, amniotic fluid, the urogenital tract, joint fluid, and various tissues. Prolonged incubation (>7 days) of these cultures may be required to recover capnocytophagae.

Capnocytophaga spp. are considered fastidious bacteria because they grow slowly on blood-enriched media and generally require an increased carbon dioxide atmosphere (5%–10%). Microaerobic and anaerobic atmospheres also have been described as conducive to isolation of these organisms from clinical samples. They grow on blood agar and often on chocolate agar but not on MacConkey or heart infusion agars. Several reports have described successfully using enriched basal media such as heart infusion, brain heart infusion with 5% blood, or basic media enriched with rabbit serum.[31,40] Other authors have reported success isolating strains from mixed cultures using selective media containing gentamicin, bacitracin, polymyxin B, vancomycin, or trimethoprim. *Capnocytophaga* isolates are reported to grow on laked blood with kanamycin and vancomycin agar medium with a reduced concentration of kanamycin (2 μg/mL), on Thayer-Martin media, on Martin-Lewis media, on Columbia agar media, and in thioglycolate broth.[66]

Colonies may be visible at 24 hours but often require 48 to 72 hours to reach 2 to 4 mm in diameter. They are convex and smooth and can show irregular edges, indicating what is described as gliding motility (Fig. 233.2). Colonies of the human oral strains can have a slight yellow pigment on initial growth, which becomes darker yellow to orange with age. Colonies are also described as having a bluish purple hue or a metallic sheen on blood agar medium.[57] Others describe colonies as being yellowish, pinkish, or bluish speckled.[66] The colonies of the zoonotic strains are not usually pigmented.

A number of methods have been employed to identify these isolates to species including conventional biochemical tests, protein profiles, multilocus enzyme electrophoresis, serotyping of IgA1 proteases, DNA probes, 16S rRNA PCR restriction fragment length polymorphism analysis, broad-range PCR from apparently nonviable organisms,[67] matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, 16S rRNA gene sequencing, and whole-genome sequencing.[17,68,69] Most current commercial identification kits are unable to identify this organism to genus and species levels. One study showed that only 11 of 24 test *Capnocytophaga* spp. were identified correctly by an automated identification system to the genus level.[70] One package insert cautions the laboratorian to consider specimen source, atmospheric preferences, Gram stain characteristics, and growth on selective agar when using their product. Table 233.1 lists some biochemical tests that can help differentiate the main *Capnocytophaga* groups. A combination of basic morphologic and conventional biochemical tests with 16S rRNA gene sequencing has proved useful in identifying these organisms to the species level.[32]

Reports have described success in identifying *Capnocytophaga* either to genus level or to genus and species level using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.[70] One study identified 22 of 24 human oral *Capnocytophaga* isolates correctly,[69] whereas another identified 22 of 23 correctly as either *C. canimorsus* or *C. cynodegmi*.[70]

FIG. 233.2 Gliding motility exhibited by *Capnocytophaga* on blood agar–based medium. *(Courtesy Dr. Edward J. Bottone.)*

THERAPY

There are no guidelines at the present time for antimicrobial susceptibility testing of *Capnocytophaga* published by either the European Committee on Antimicrobial Susceptibility Testing or the Clinical and Laboratory Standards Institute. The inherent slow growth of *Capnocytophaga* spp. isolates makes antibiotic susceptibility difficult to perform. Owing to the lack of a standardized method, many different susceptibility assays have been published for these organisms including agar dilution,[71–75] broth microdilution,[76,77] Etest,[78] and agar diffusion.[79] These antimicrobial susceptibility tests also used different media, incubation times, and atmosphere of incubation (anaerobic vs. increased carbon dioxide) (5%–10%)[77–82]; thus for certain drugs (see further on), results among individual laboratories may not agree.

Early reports of antibiotic susceptibility testing revealed almost universal susceptibility to the β-lactam antibiotics (Table 233.2).[71,75,76] Before 1987, there were only four reported β-lactamase strains among *Capnocytophaga* spp.[19,76] However, the detection of β-lactamase production became more common as studies were published in the 1980s and 1990s.[73,81,83,84] Aside from being resistant to both penicillin and amoxicillin, many of these isolates showed resistance to third-generation cephalosporins such as ceftazidime and cefotaxime. They remained susceptible to β-lactam/β-lactamase inhibitor combinations such as amoxicillin-clavulanate, piperacillin-tazobactam, and ampicillin-sulbactam.[72–74,82,84] It was not unusual for more than 50% of the published study isolates to produce a β-lactamase.[19,72] One study reported that the ceftazidime resistance of 195 human oral *Capnocytophaga* spp. ranged from 37% to 100% in the years 1993–2002.[24]

Investigators soon discovered that the *Capnocytophaga* isolates produced several different types of β-lactamases including the newly discovered *Capnocytophaga sputigena* 1 (CSP-1) β-lactamase.[80–82] CSP-1 is associated with resistance to both the penicillins (amoxicillin, piperacillin) and some second-generation and third-generation cephalosporins (cefotaxime, ceftazidime) but is inhibited by clavulanic acid. Thus CSP-1-harboring *Capnocytophaga* organisms are still highly susceptible

TABLE 233.2 Antibiotic Susceptibilities of *Capnocytophaga*

HIGHLY SUSCEPTIBLE	INTERMEDIATELY SUSCEPTIBLE[b]	RESISTANT OR INCONSISTENT
Imipenem[19,72,77,82,87]	Ciprofloxacin[71,73,74,80,79]	Gentamicin[73,74,88]
Meropenem[91]	Clindamycin[73,75,77,79,80,84]	Vancomycin[75]
Piperacillin/tazobactam[72,82]	Erythromycin[75,80,84]	Polymyxin B[74,80]
Amoxicillin/clavulanate[16,82,84]	Tetracycline[75,84]	Trimethoprim[71]
Tigecycline[88]	Aztreonam[71,72,82]	Ceftazidime[16,19,24,77,84] Cefotaxime[16,19,77,84] Amoxicillin[16,72,82,84,87]

[a]These susceptibility groups are predominantly useful for treating human oral *Capnocytophaga* spp.; zoonotic *Capnocytophaga* spp. remain more susceptible to antibiotics.
[b]It is recommended to perform antibiotic susceptibility in relation to these drugs before using them to treat patients infected with *Capnocytophaga* spp.

in vitro to β-lactamase inhibitor combinations such as amoxicillin-clavulanate and piperacillin-tazobactam. In addition, organisms producing CSP-1 are still susceptible to the carbapenem imipenem. Other β-lactamases commonly found in oral *Capnocytophaga* spp. are CfxA2 and CfxA3 β-lactamases. According to one study, these class A β-lactamases may be responsible for up to 70% to 80% of β-lactam resistance in *Capnocytophaga* spp.[84,85]

Both CfxA2 and CfxA3 β-lactamases are predominately cefuroximases that hydrolyze penicillins and extended-spectrum cephalosporins but are still susceptible to inhibition by clavulanic acid.[84] CfxA2 and CfxA3 β-lactamases may be chromosomal or plasmid based.[86] Different variants of CfxA have been described.[86] Other β-lactamases produced by *Capnocytophaga* include the extended-spectrum M-17 derived from *bla*TEM_{-1a}. As with many other extended-spectrum β-lactamases,[87] this enzyme is able to effectively hydrolyze both ceftazidime and cefotaxime, although cefotaxime is less affected. The rare chromosome-based *bla*OXA-347 has been described in *Capnocytophaga* isolates using whole-genome sequencing; this carbapenemase has been detected in *C. cynodegmi* chromosomes and is responsible for resistance to imipenem and to second-generation and extended-spectrum cephalosporins.[88]

For other non–β-lactam drugs, the resistance patterns depend on the specific drug. Most *Capnocytophaga* organisms are resistant to gentamicin,[73,88] polymyxin B,[74,80] and trimethoprim.[71] However, for drugs such as gentamicin, the minimal inhibitory concentrations range from being highly susceptible levels (0.05 μg/mL)[77,80] to complete high-level resistance (128 μg/mL).[73,74,88] These data may indicate a lack of standardization among the various described methods.[89]

For clindamycin, some studies indicate rare resistance,[73,75,77,79,80] but others note an increase in resistance approaching 30%.[84] Erythromycin resistance seems to parallel clindamycin resistance.[75,80,84] Resistance to both of these drugs is associated with the *erm*(F) and *erm*(C) genes.[81] Quinolone resistance is also becoming more common[77,79] even though early reports[71,73,74,76,77,79] indicated a lack of resistance to this class of drugs. Tetracycline resistance is not common in published studies.[75,80,84]

For oral therapy of milder infections, clindamycin, doxycycline, or a fluoroquinolone can be used. Carbapenems may be indicated in mixed soft tissue infections with more resistant organisms than *Capnocytophaga* spp. Aminoglycosides, antistaphylococcal penicillins, colistin, and first-generation cephalosporins are not considered useful.

Initial therapy in most *Capnocytophaga* cases is empirically determined and involves treatment with broad-spectrum antibiotics based on presenting diagnosis. When either a gram-negative rod or a *Capnocytophaga* sp. is suspected, more specific antimicrobial treatment is prescribed. Because it may take several days before a *Capnocytophaga* sp. can be presumptively recognized, clinicians should keep this organism in mind in cases in which there is a history of dog or cat bite or with neutropenic patients who have oral mucositis or periodontitis. This is especially true if the patient is asplenic or functionally asplenic or has a history of alcohol abuse.

Differences in outcome of serious *Capnocytophaga* infections have been documented. In a Denmark study of 39 cases of dog bites or associated with dog bites, 12 patients died.[60] All but 2 of the 39 patients were infected with *C. canimorsus* and were treated with penicillin, which may be an inferior drug therapy. No data on β-lactamase positivity rates were provided, even though to our knowledge, no β-lactamase–producing *C. canimorsus* organisms have been detected.

In a study of 11 cases of *Capnocytophaga* bacteremia,[19] *C. ochracea* and *C. sputigena* were important causes of fever in neutropenic patients, especially where β-lactam therapy has failed. In this study, eight patients were infected with β-lactamase–positive *Capnocytophaga*. In these cases, the introduction of imipenem would be the preferred option. A second option would be a fluoroquinolone or clindamycin.

Another study of a series of 28 patients with cancer concluded that *Capnocytophaga* bacteremia is usually an uncomplicated condition.[90] The outcomes were good after antibiotic therapy; however, only one of their isolates produced a β-lactamase even though 50% of their tested isolates were resistant to ciprofloxacin. Consequently, the authors recommended that these cases be managed with a carbapenem or a ureidopenicillin with a β-lactamase inhibitor.

Clinicians should be aware of the increased β-lactam resistance of *Capnocytophaga* to second-generation and extended-spectrum cephalosporin antibiotics. A case of *C. sputigena* fatal bacteremia was described in which the patient was treated with both amikacin and ceftazidime, to which the organisms were resistant.[81] Another case of CfxA3 β-lactamase–positive *C. sputigena* in an immunocompromised pediatric patient has been described.[91] Treatment was altered to both meropenem and clindamycin, and the patient recovered. It may be difficult to obtain a *Capnocytophaga* susceptibility test in a timely manner, as most laboratories have not validated this organism for susceptibility testing and would send out to a reference laboratory for assays of this type. Etest incubated in increased carbon dioxide or anaerobically may be the easiest test to perform, as very few laboratories perform agar dilution.

PREVENTION

Clinician awareness and patient education are the most effective ways to prevent *Capnocytophaga* infections. Clinicians should keep in mind patient risk factors associated with these infections. Clinical laboratories should know how to handle specimens and identify isolates effectively and quickly when *Capnocytophaga* organisms are suspected. This awareness can reduce the time to diagnosis and result in more specific antimicrobial treatment. Rapid presumptive diagnosis may result in the patient receiving better targeted antibiotics for these organisms before a conclusive diagnosis is confirmed. Individuals with enhanced susceptibility to infection, particularly patients who have undergone splenectomy or are asplenic, should be made aware of activities such as pet (dog or cat) ownership that increase their risk for developing *Capnocytophaga* sepsis.[38,92,93] Asplenic patients should not take care of a neighbor's dog or cat.[94] Several authors suggest the controversial step of clinicians prophylactically treating all patients in high-risk groups for possible *Capnocytophaga* infections after a dog bite.[2] One group is reportedly working on developing a standard test to detect more dangerous strains,[36] whereas another group is trying to understand why some individuals are more prone to developing overwhelming disease.[38,74]

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The complete reference list is available online at Expert Consult.

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234 *Bartonella*, Including Cat-Scratch Disease

Stacey R. Rose and Jane E. Koehler[a]

SHORT VIEW SUMMARY

Epidemiology and Microbiology

- Fastidious, facultatively intracellular, pleomorphic gram-negative bacilli
- Transmission via infected feces of arthropod vectors by direct inoculation into nonintact human skin
- *Bartonella bacilliformis:* endemic to Andes mountains; reservoir—humans; vector—sand flies
- *Bartonella henselae:* globally endemic; reservoir—cats; vector—cat flea; transmitted to humans via cat scratch and inoculation of wound with flea feces
- *Bartonella quintana:* sporadic outbreaks worldwide; associated with homelessness and other conditions of poor sanitation; reservoir—humans; vector—human body louse; transmitted to humans via inoculation of louse feces into louse bite wound

Clinical Features

- Chronic bloodstream infections (for months) is a hallmark of *Bartonella* infections
- *B. bacilliformis:* in immunocompetent patients causes biphasic illness—Oroya fever (acute phase) characterized by fever, hemolytic anemia and high fatality rate when untreated; verruga peruana (late phase), characterized by crops of vascular proliferative skin lesions that evolve through stages
- *B. henselae*: in immunocompetent patients causes cat-scratch disease (CSD)—self-limited regional lymphadenopathy, more common in pediatric population and in warm, humid climates that favor feline flea infestation
- *B. henselae:* in advanced human immunodeficiency virus (HIV) infection and other immunocompromising conditions, causes vascular proliferative lesions—bacillary angiomatosis (BA) and hepatic and splenic bacillary peliosis (BP)
- *B. henselae:* blood culture-negative endocarditis—usually occurs in patients with preexisting native valve lesion or prosthetic valve; may be associated vasculitis/glomerulonephritis, including positive immune markers (cytoplasmic-antineutrophil cytoplasmic antibody (c-ANCA) and/or anti–proteinase 3 antibodies (anti-PR3)
- *B. henselae:* in immunocompetent and immunocompromised patients (HIV-infected and transplant recipients) causes fever of unknown origin (FUO), disseminated infection
- *B. quintana:* in immunocompetent homeless patients causes trench fever—usually self-limited febrile illness with bacteremia
- *B. quintana:* blood culture-negative endocarditis—can occur in patients with normal heart valves; may be associated with c-ANCA– or anti-PR3–positive vasculitis/glomerulonephritis
- *B. quintana:* in advanced HIV infection and other immunocompromising conditions causes vascular proliferative lesions—BA, usually cutaneous, subcutaneous, osseous (but not hepatic)
- *B. quintana:* in immunocompromised HIV-infected (but not transplant) patients causes FUO, disseminated infection

Diagnosis

- Extremely fastidious and difficult to culture
- Serology (indirect immunofluorescence assay [IFA])—mainstay for diagnosis, but cross-reactivity among *Bartonella* spp. common, and IFA may be negative for weeks early in disease course; sensitivity is highest in immunocompetent patients with CSD; slightly lower in solid-organ transplant (SOT) recipients and lowest in late-stage acquired immunodeficiency syndrome patients
- Histopathology: CSD—granulomatous inflammation; BA/BP—vascular proliferation; Warthin-Starry or Steiner stain demonstrates *Bartonella* bacilli in tissue, especially in BA/BP lesions
- Polymerase chain reaction amplification of DNA extracted from tissue or blood increasingly important and available

Therapy

- *B. bacilliformis:* Oroya fever, first line—ciprofloxacin plus ceftriaxone for 14 days, although fluoroquinolone resistance has been reported; alternative: chloramphenicol, coadministered with a β-lactam antibiotic
- *B. bacilliformis:* verruga peruana, first line—azithromycin for 7 to 14 days; alternatives—rifampin for 21 to 28 days or ciprofloxacin for 14 days
- *B. quintana* and *B. henselae:* infection in immunocompromised hosts (e.g., in HIV-infected and SOT recipients with BA/BP)—doxycycline is first line; erythromycin or azithromycin is second choice (doxycycline is preferred due to tolerability issues and drug-drug interaction potential with macrolides); duration of 3 months or longer for cutaneous BA disease and 6 months or longer for visceral, extensive cutaneous, or relapsing *Bartonella* infection
- *B. quintana:* bacteremia without endocarditis (trench fever)—doxycycline for 4 weeks plus gentamicin for the first 2 weeks
- *Bartonella* spp.: endocarditis—doxycycline for 6 weeks plus gentamicin for first 2 weeks *OR* both doxycycline plus rifampin for at least 6 weeks (doxycycline + rifampin preferable to doxycycline plus gentamicin in setting of *Bartonella* endocarditis-associated renal insufficiency, which occurs frequently)
- *B. henselae:* CSD lymphadenopathy in immunocompetent host—no treatment (self-limited); possibly azithromycin for extensive CSD lymphadenitis
- *B. henselae:* CSD disseminated or CNS infection in immunocompetent host (e.g., encephalitis, neuroretinitis, hepatosplenic involvement, osteomyelitis)—two-agent therapy required, doxycycline (or azithromycin) +/– rifampin for 4 to 6 weeks

Prevention

- *B. henselae:* control of flea infestation of cats is most important; avoid cat scratches of humans
- *B. quintana:* eradicate and prevent body lice infestation of humans

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BACKGROUND AND CLASSIFICATION OF *BARTONELLA* SPECIES

The genus *Bartonella* is a member of the class Alphaproteobacteria and family Bartonellaceae, and it is closely related to the genera *Brucella* and *Agrobacterium*; members of the family Rickettsiaceae are more distantly related. On the basis of genetic similarity,[1,2] unification of the genera *Bartonella* and *Rochalimaea* as a single genus and the removal of the family Bartonellaceae from the order Rickettsiales were proposed in 1993.[2] The similarity of *Bartonella* to the pathogen *Brucella* has been further substantiated through whole-genome sequencing showing that *Bartonella* contains a reduced version of the chromosomal elements of *Brucella melitensis*.[3]

The genus *Bartonella,* synonymous with *Bartonia,* was described in 1913 and referred to the human erythrocyte-adherent organisms originally described by Dr. A. L. Barton in 1909.[4,5] The type species is *Bartonella bacilliformis.* Limited to the Andes mountain regions of South America, *B. bacilliformis* infection received little attention outside its endemic zone until related bacteria, originally classified in the genus *Rochalimaea,* were found to be pathogens in individuals with acquired immunodeficiency syndrome (AIDS) in the early 1990s.

The former genus *Rochalimaea,* previously grouped with *Bartonella* in the order Rickettsiales, had long contained only two member species: *Rochalimaea vinsonii,* the "Canadian vole agent," and *Rochalimaea quintana*,[6] the agent of trench fever, a debilitating but self-limited human illness so named after it affected numerous military personnel in World War I.[7–9] Except for sporadic outbreaks, trench fever was rarely reported after World War I. However, *R. quintana* reemerged in the 1990s as a pathogen causing a debilitating and fatal disease in patients with AIDS and in urban homeless individuals.[10–13,14] Two new species pathogenic to humans, originally named *Rochalimaea henselae* and *Rochalimaea elizabethae,* also were identified.[15–18]

In 1995 a further merger of species in the genus *Grahamella* (also erythrocyte-associated bacteria, infecting rodents, birds, fish, and other animals) into the genus *Bartonella* was proposed.[19] Numerous *Bartonella* spp. and subspp. have been identified subsequently,[20–30] and some of these *Bartonella* spp. cause infrequent infections in humans.[28–36] Although *Bartonella* spp. have been characterized recently as "emerging" pathogens, DNA analysis of dental pulp from ancient human remains provides evidence that *B. henselae* and *B. quintana* have infected humans since antiquity.[37]

A list of validated members of the genus *Bartonella* is provided in Table 234.1. A continuously updated list of validated *Bartonella* spp. can be found at http://www.bacterio.net/bartonella.html.

TABLE 234.1 Currently Recognized *Bartonella* Species and Their Identified Potential as Human Pathogens

Documented as Common Human Pathogens
Bartonella bacilliformis
Bartonella henselae
Bartonella quintana
Uncommon or Suspected as Human Pathogens
Bartonella alsatica
Bartonella ancashensis
Bartonella clarridgeiae
Bartonella doshiae
Bartonella elizabethae
Bartonella grahamii
Bartonella koehlerae
Bartonella rochalimae
Bartonella schoenbuchensis
Bartonella tribocorum
Bartonella vinsonii subsp. *arupensis*
Not Identified as Human Pathogens
Bartonella acomydis
Bartonella apis
Bartonella birtlesii
Bartonella bovis
Bartonella callosciuri
Bartonella capreoli
Bartonella chomelii
Bartonella coopersplainsensis
Bartonella florencae
Bartonella fuyuanensis
Bartonella heixiaziensis
Bartonella jaculi
Bartonella japonica
Bartonella pachyuromydis
Bartonella peromysci
Bartonella queenslandensis
Bartonella rattaustraliani
Bartonella senegalensis
Bartonella silvatica
Bartonella talpae
Bartonella taylorii
Bartonella vinsonii subsp. *berkhoffii*
Bartonella vinsonii subsp. *vinsonii*

EPIDEMIOLOGY OF THE COMMON HUMAN-PATHOGENIC *BARTONELLA* SPECIES

Bartonella spp. are primarily infectious agents of nonhuman mammals. Humans are incidental hosts for most *Bartonella* spp., with stercorarian transmission via inoculation of *Bartonella*-infected feces excreted from arthropod vectors into nonintact human skin. The exceptions are *B. quintana* and probably *B. bacilliformis*, for which the definitive mammalian reservoir is believed to be humans.

Bartonella bacilliformis Epidemiology

Natural transmission of *B. bacilliformis* infection is mainly confined to the Andes mountain range in Peru, Ecuador, and Colombia at altitudes between 500 and 3200 m.[38] This is presumed due to the limited regional distribution of the sand fly vectors (genus *Lutzomyia* [formerly *Phlebotomus*]) of *B. bacilliformis*. Even in the modern antibiotic era, focal outbreaks continue,[39] and new geographic areas of *B. bacilliformis* infection have been reported recently.[39] In addition to outbreaks in new geographic areas, outbreaks in areas of endemicity continue to occur; for instance, 191 cases were reported from 2003 in a community with endemic disease in Peru.[40] Cases of *B. bacilliformis* infection, one possibly from vertical transmission and the other after blood transfusion, have been reported.[41,42] No nonhuman vertebrate reservoirs have been identified for *B. bacilliformis.*

Bartonella quintana Epidemiology

B. quintana is globally distributed, and the only identified vector of *B. quintana* is *Pediculus humanus,* the human body louse. Outbreaks of trench fever (also known as Wolhynia fever, Meuse fever, His-Werner disease, shin bone fever, shank fever, and quintan or 5-day fever) have been focal and widely separated. Clusters of trench fever cases usually are associated with conditions of poor sanitation and personal hygiene and are significantly associated with exposure to body lice.[43] A survey of published reports of zoonotic and vector-borne infections, as assessed by seroprevalence in the homeless and very poor in the United States and Europe from 1990–2014, identified *B. quintana* as the most commonly reported vector-borne infection in the homeless.[44] The prevalence of *B. quintana* in body lice can be high: *B. quintana* was detected in 33.3% of body lice pools removed from infested homeless persons in San Francisco.[45] *B. quintana* has been isolated from captive nonhuman primates in China and the United States and, more recently, from wild-caught Japanese macaques, suggesting that nonhuman primates may serve as a natural reservoir for *B. quintana*.[46–48]

Bartonella henselae Epidemiology

B. henselae is globally endemic and has been detected in 800-year-old French cats[49]; serologic studies indicate that infection of domestic cats occurs worldwide, with the prevalence of antibodies in cats being higher in warm, humid climates, where fleas are more prevalent.[50] Prevalence of bacteremia in cats varies[51,52–54] but tends to be higher among feral animals in any particular locale. The *B. henselae* colony-forming units (CFU)/mL of blood in infected cats can reach extremely high levels of