204 *Corynebacterium diphtheriae* (Diphtheria)

Paul G. Saleeb

SHORT VIEW SUMMARY

Definition

- *Corynebacterium diphtheriae* is a gram-positive bacillus that causes toxic upper respiratory disease or cutaneous lesions in susceptible hosts.

Epidemiology

- *C. diphtheriae* is transmitted via respiratory or cutaneous secretions. Asymptomatic carriers and, less commonly, contaminated fomites serve as reservoirs for infection. The incidence of diphtheria has precipitously declined with widespread use of the toxoid vaccine. Persons at risk for disease are those with a history of inadequate vaccination who have been exposed to an endemic or epidemic setting.

Microbiology

- *C. diphtheriae* is a gram-positive, unencapsulated, club-shaped, aerobic bacillus that is subdivided into four biovars. The organisms display "Chinese character" arrangement on Gram stain and form black colonies with brown halos on tellurite-containing culture media. The diphtheria exotoxin is responsible for the disease's cardiac and neurologic manifestations.

Clinical Manifestations

- Pharyngeal diphtheria presents with fever, sore throat with an associated pseudomembrane, submandibular edema, and cervical lymphadenopathy, resulting in a "bull neck" appearance. Pseudomembranous extension and obstruction of the airways may lead to respiratory failure. Cardiac and neurologic toxicities are complications of severe disease. Cutaneous diphtheria classically presents as an ulcerative lesion and is not typically associated with toxicity.

Diagnosis

- A clinical diagnosis can be made in an individual with appropriate epidemiologic risk factors presenting with pharyngitis complicated by pseudomembrane formation and cardiac or neurologic toxicity. A definitive diagnosis is made by culturing respiratory or cutaneous specimens on tellurite-containing media and performing additional biochemical testing. Polymerase chain reaction or enzyme immunoassay can be used to detect the diphtheria toxin.

Therapy

- Equine diphtheria antitoxin should be administered to all persons with suspected diphtheria. A 14-day course of antibiotic therapy with erythromycin or procaine penicillin G prevents further elaboration of toxin and transmission to susceptible hosts. All individuals should be vaccinated during convalescence, as infection may not induce a protective level of immunity.

Prevention

- A primary series of vaccination with diphtheria toxoid affords protective immunity in children and adults. To counter the effect of waning immunity, booster doses should be administered every 10 years to older children and adults who have completed the primary vaccination series. Close contacts and *C. diphtheriae* carriers should be treated with a course of antibiotics until microbiologic clearance is documented and should also undergo primary or booster vaccination as necessary.

A primary series of vaccination with diphtheria toxoid affords protective immunity in children and adults. To counter the effect of waning immunity, booster doses should be administered every 10 years to older children and adults who have completed the primary vaccination series. Close contacts and *Corynebacterium diphtheriae* carriers should be treated with a course of antibiotics until microbiologic clearance is documented and should also undergo primary or booster vaccination as necessary.

Diphtheria was one of the most lethal diseases of childhood until the middle of the 20th century. Scientific study of the disease and its causative pathogen, *C. diphtheriae*, led to seminal discoveries and significant advancement in the fields of bacteriology, immunology, and vaccine science. A precipitous decline in the incidence of disease has occurred worldwide since the introduction of the toxoid vaccine into routine immunization schedules. However, the 1990s epidemic in the former Soviet Union republics reinforces the crucial need to maintain robust immunization programs.

HISTORY

The earliest written records of diphtheria, some of which are attributed to Hippocrates, can be traced back to the Hellenic period during the 5th or 4th century BC. Vivid depictions of diphtheria were also documented during the Renaissance: Bartholin described the disease as "angina puerorum" and "morbus strangulatorius," evoking its propensity to cause disproportionate morbidity in young children.[1] Outbreaks occurred sporadically in Spain during the 1600s, and a major epidemic broke out in New England from 1735–40, decimating the population of children in several towns. Belief that divine retribution was the cause of the outbreak may have contributed to the Great Awakening of the mid-1700s.[2] During an 1818–20 epidemic in Tours, Bretonneau described diphtheria's salient clinical findings and differentiated it from other causes of "throat distemper." He named the disease "diphtheria" (from the Greek "diphthera," meaning "leather hide"), aptly depicting its characteristic pseudomembrane. He may have also been the first clinician to perform a tracheostomy successfully for pharyngeal diphtheria in 1825.[3]

Understanding of the etiology, pathogenesis, and treatment of diphtheria advanced significantly as discoveries were made in the late 1800s and early 1900s. Klebs isolated *C. diphtheriae* from a pseudomembrane in 1884, and Loeffler proved it to be the etiologic agent of diphtheria. In 1888 Roux and Yersin discovered the exotoxin and described its clinical effects.[3] The treatment of diphtheria advanced significantly in 1890 when von Behring and Kitasato developed antitoxin in guinea pigs, demonstrating the concept of passive immunity. For this seminal discovery von Behring was awarded the inaugural Nobel Prize for Physiology and Medicine in 1901.[4] A combination toxin-antitoxin vaccine was initially used for prevention of diphtheria, until

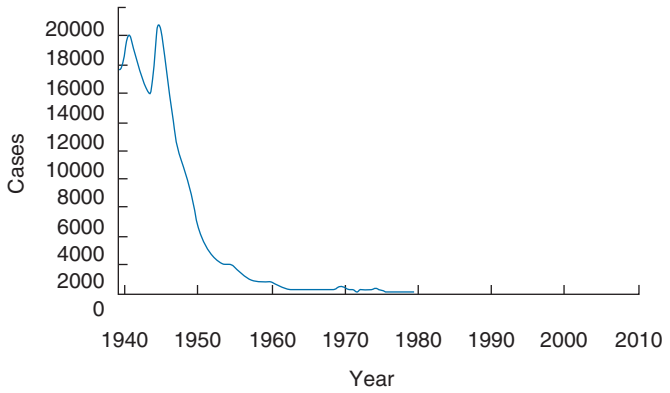


FIG. 204.1 Diphtheria: United States, 1940–2011. *(From Centers for Disease Control and Prevention. Diphtheria. In: Hamborsky J, Kroger A, Wolfe S, eds.* Epidemiology and Prevention of Vaccine-Preventable Diseases. *13th ed. Washington DC: Public Health Foundation; 2015:107–118.* https://www.cdc.gov/vaccines/pubs/pinkbook/dip.html.*)*

Ramon developed a safe and immunogenic heat- and formalin-inactivated toxoid vaccine in 1923. Aluminum salt adjuvants were incorporated into the toxoid vaccine to increase its immunogenicity, and by the 1940s an effective vaccine had been developed. Cases of diphtheria in the United States decreased by over 99% from approximately 206,000 cases in 1921 to 5 reported cases since 2000 (Fig. 204.1).[5,6] In the 1990s, however, there was a resurgence of diphtheria in the former Soviet Union republics, as their health care system was disrupted. At the peak of the epidemic there were more than 140,000 cases and approximately 4000 deaths.[7] Targeted vaccination campaigns and a coordinated international effort halted the outbreak.

THE PATHOGEN

The genus *Corynebacterium* is grouped within the order Actinomycetales and consists of more than 80 species, several of which are medically important. *C. diphtheriae* (from "korune" and "diphthera," Greek for "club" and "leather," respectively) is named for its characteristic clubbed-shape appearance on Gram stain and its propensity to form a leather-like pseudomembrane. The organism is a gram-positive, unencapsulated, nonmotile, nonsporulating, aerobic rod. On Gram stain the bacilli display a characteristic "Chinese characters" arrangement and may even appear gram variable, due to thinning of the cell wall leading to decolorization of stain.[8] Metachromatic granules containing polyphosphate are found in the polar regions of the bacterium and appear bluish-purple to red when stained with methylene blue. Loeffler developed his eponymous culture medium containing dextrose, horse serum, and beef heart infusion for isolation of *C. diphtheriae*. Due to the tendency for overgrowth of commensals on Loeffler medium, more selective media containing telluric acid (e.g., Mueller-Miller, Tinsdale) were later developed.[9] On Tinsdale medium potassium tellurite inhibits gram-negative organisms and most upper respiratory flora; *C. diphtheriae* and *C. ulcerans,* another medically important *Corynebacterium* sp., appear as grayish-black colonies with a surrounding brown halo. Urease testing can distinguish between the two organisms, as *C. diphtheriae* is urease negative, whereas *C. ulcerans* is urease positive.

C. diphtheriae is subdivided into four biovars (biotypes): *belfanti*, *gravis*, *intermedius*, and *mitis*. An individual may harbor more than one biovar concurrently. Although clinically similar, biovars may be distinguished on the basis of colony morphology, hemolysis, biochemical reactions (e.g., API Coryne:; bioMérieux, La Balme Les Grottes, France), and, more recently, 16S ribosomal RNA sequencing.[10,11] Ribotyping and pulsed-field gel electrophoresis have been used to type *C. diphtheriae* strains during outbreaks and for surveillance purposes; these methods have been supplanted by multilocus sequence typing due to its improved reproducibility.[12,13]

In the late 19th century Loeffler discovered the presence of avirulent, nontoxigenic strains of *C. diphtheriae* in healthy carriers and noted that these strains are morphologically indistinguishable from toxigenic strains. Corynebacteriophages carry *tox*, the gene for exotoxin production, and convert strains of *C. diphtheriae* from nontoxigenic to toxigenic via a lysogenic cycle. Expression of *tox* is regulated by DtxR, an iron-activated repressor that is derepressed in low iron states. The potent diphtheria toxin is composed of three domains: a cell receptor binding domain, a transmembrane domain, and a catalytic N-terminal adenosine diphosphate (ADP)-ribosyltransferase domain.[8] *C. pseudotuberculosis* and *C. ulcerans* also elaborate the diphtheria toxin; both species may be differentiated from *C. diphtheriae* by means of biochemical testing.[10,14] Laboratory methods for detection of toxin include polymerase chain reaction (PCR), enzyme immunoassay (EIA), and immunochromatography.[14,15]

EPIDEMIOLOGY

The role of the asymptomatic carrier as a reservoir for infection was first recognized in the late 1800s.[16] Humans were originally thought to be the only reservoir, but *C. diphtheriae* has now been isolated from horses, cattle, and domestic cats.[17–19] Although diphtheria has been classically understood as an upper respiratory disease acquired via inhalation of infected droplets, cutaneous lesions may provide a more efficient means of transmission, resulting in both respiratory and cutaneous diphtheria. Cutaneous lesions are probably the major reservoir for infection in resource-limited environments, serving as a source of natural immunity in these settings.[20] Transmission of infection between skin lesions and between the respiratory tract and skin lesions (bidirectional) has been documented.[21] Cases of reinfection, probably due to transmission via contaminated fomites, have also been described.[22]

Young children suffered disproportionately from diphtheria during the prevaccine era; up to 70% of cases occurred in children younger than 15 years.[20] In the New England epidemic of 1735–40, 40% of all children below 10 years of age died in a single year in Hampton Falls, New Hampshire. In 1881 greater than 1% of deaths in children younger than 10 years in New York were caused by diphtheria.[23] Children ages 5 to 14 experienced high attack rates of up to 683 per 100,000 population from 1921–24 in Baltimore; the case fatality rate in that city ranged from 5% to 8%.[24] Epidemics tended to peak in the fall at the beginning of the school year and affected those at the age of school entry. In England in the late 1930s diphtheria was the second most common cause of mortality in children, after pneumonia, causing 32 deaths per 100,000 population in those younger than 15 years.[25]

Although introduction of antitoxin treatment in the early 1900s had a beneficial effect on mortality, incidence rates of disease remained elevated. In the immediate prevaccine era the incidence rate in the United States was 237 per 100,000 population per year.[26] Incidence rates decreased worldwide from the 1930s onward, coincident with the introduction of routine toxoid vaccination in children. During World War II, however, outbreaks occurred in European countries that had experienced decreasing rates of infection in the previous years. German soldiers infected with *C. diphtheriae* biovar *gravis* reintroduced the disease in several occupied territories.[25] Unlike earlier epidemics in which younger children were disproportionately affected, a relatively high percentage of older children and adults contracted disease, likely due to wartime displacement of large populations and improved living conditions over the preceding decades, leading to decreased exposure of younger children to infection.[20] After World War II incidence rates in industrialized countries declined precipitously as childhood immunization programs were strengthened. By 1965 the attack rate in the United States had declined to 0.08 per 100,000 population.[27] Isolated outbreaks did occur among minority and indigent groups; unimmunized individuals constituted the majority of these cases. One such example was the 1970s outbreak in the Skid Road neighborhood of Seattle, which was concentrated in Native American men with high rates of alcohol dependence and was characterized by a predominance of cutaneous diphtheria.[22,28]

A major epidemic of diphtheria occurred in the former Union of Soviet Socialist Republics (USSR) in the early 1990s. The incidence rate in the USSR was as low as 0.04 per 100,000 population in the mid 1970s. By the 1980s, however, a combination of factors contributed to the 1990s epidemic: decreased rates of immunization in children due to vaccine shortages and antivaccine propaganda, vaccination of children with the reduced-dose adult formulation of diphtheria toxoid, waning adult immunity, and transmission of infection from unvaccinated

members of the military returning from Afghanistan.[23] After the breakup of the USSR in 1991 the health care system collapsed, and the incidence of diphtheria spiked to as much as 50,000 cases in 1995. The predominant strain in Russia, Ukraine, Belarus, the Baltic republics, and northern Kazakhstan was *gravis*, whereas *mitis* was predominant in southern Kazakhstan, Tajikistan, Uzbekistan, and Kyrgyzstan. In Russia, where the majority of cases occurred, incidence rates in adolescents and adults were more than 20 per 100,000 population.[25] Mobilization of an intensive vaccination campaigns in coordination with World Health Organization (WHO) and the United Nations Children's Fund brought about an end to the epidemic in the late 1990s.

Global incidence rates of diphtheria have declined steadily as a result of widespread implementation of the WHO's Expanded Programme on Immunization (EPI). From 1980 to 2000 there was a greater than 90% decrease in global incidence rates. Vaccination has prevented an estimated 40 million cases of diphtheria.[26,29] Despite these impressive figures, outbreaks continue to occur in resource-limited settings, highlighting gaps in vaccination coverage. India is currently the country with the greatest number of cases worldwide. In northern Kerala there were 533 cases in 2016, the majority of which occurred in individuals older than 10 years.[30,31]

In the prevaccine era placental passage of maternal antibodies afforded passive immunity to neonates. As passive immunity waned at approximately 6 months of age, active immunity developed naturally by means of disease or through clinically silent infection; the majority of children had developed immunity to diphtheria by age 15 years.[32] After the introduction of childhood vaccination, however, the burden of disease shifted to susceptible older children and adults. Examples were clearly seen in Jordan and Sudan, where younger children were mostly affected during epidemics before the implementation of effective childhood vaccination programs, but adults and older children were disproportionately affected during outbreaks occurring after the institution of these programs.[33,34] Waning immunity to diphtheria underpins the rationale for booster vaccination of older children and adults.

With the decline of disease due to toxigenic *C. diphtheriae*, the pathogenicity of nontoxigenic strains is becoming increasingly recognized. The *tox* gene is not necessary for the life cycle or metabolism of *C. diphtheriae*; immunized individuals are more likely to harbor nontoxigenic strains, which are consequently more likely to circulate in the community.[28] Nontoxigenic *C. diphtheriae* has been associated with blood stream infections and endocarditis in individuals with comorbid conditions, such as alcoholism, dental disease, and intravenous (IV) drug use.[35–37] The majority of nontoxigenic strains do not carry the gene *tox*; however, nontoxigenic strains that harbor the gene without expressing the protein exotoxin have recently been detected.[38]

PATHOGENESIS

Although the diphtheria exotoxin is responsible for much of the disease manifestations, other virulence factors also play a role in pathogenesis. Neuraminidase and *trans*-sialidase facilitate binding to the host cell and allow the organism to scavenge sialic acid for nutrition and metabolism.[8,39] SpaA, SpaD, and SpaH pili mediate adherence to pharyngeal, laryngeal, and respiratory epithelial cells.[40,41] DIP0733 and DIP2093 adhesins facilitate binding to the extracellular matrix, adhesion and invasion of epithelial cells, biofilm formation, and survival in macrophages.[42–45] Both toxigenic and nontoxigenic strains are capable of converting fibrinogen to fibrin, a finding that may explain the rare occurrence of pseudomembranes in the setting of nontoxigenic *C. diphtheriae* infection.[46]

The diphtheria exotoxin, containing 535 amino acids, is composed of two covalently bonded fragments, fragment A and fragment B. Fragment B contains the receptor binding and transmembrane domains, which allow cell surface binding and transport into the cytosol, respectively. Fragment A, containing the catalytic domain, facilitates ADP-ribosylation and consequent irreversible inhibition of elongation factor 2, a eukaryotic protein necessary for the coordinated movement of transfer RNA, messenger RNA, and the ribosome during the elongation cycle of protein synthesis.[8,47] One molecule of the exotoxin is sufficient to kill a cell; the lethal dose in humans may be as little as 100 ng/kg.[48,49]

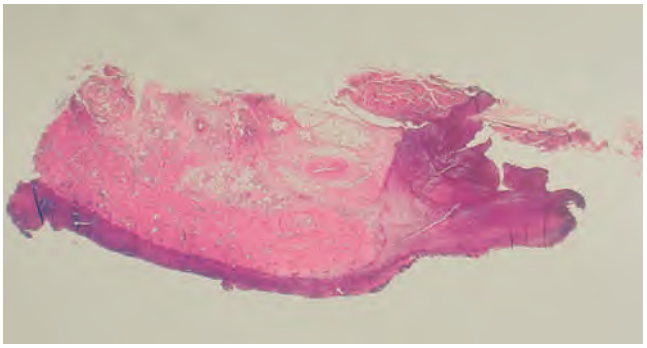


FIG. 204.2 Pharyngeal pseudomembrane. Epithelium is absent; at one side, inflammatory exudate extends to underlying muscle. Hematoxylin and eosin staining. Original magnification ×2.5. *(From Hadfield TL, McEvoy P, Polotsky Y, et al. The pathology of diphtheria.* J Infect Dis. *2000;181(suppl 1):S116–S120; with permission of Oxford University Press.)*

C. diphtheriae infection leads to mucosal edema with subsequent necrosis and ulceration. A fibrinous exudate overlying the desquamated mucosae forms the adherent pseudomembrane. On histopathology the pseudomembrane consists of fibrin and denuded epithelial cells with an associated neutrophilic infiltrate and clusters of *C. diphtheriae* organisms (Fig. 204.2). The pseudomembrane may extend to form a cast of the upper airways. Forcible removal of the pseudomembrane may cause bleeding; dislodgement may lead to aspiration and asphyxiation.[50] Edematous cervical, parabronchial and mediastinal lymph nodes often hemorrhage or necrose.[51]

CLINICAL MANIFESTATIONS

C. diphtheriae usually causes upper respiratory tract or cutaneous disease. Cardiac and neurologic complications are the most frequent toxin-mediated manifestations. Both toxigenic and nontoxigenic strains may rarely disseminate to distant sites.

Respiratory Tract Diphtheria

Clinical signs and symptoms of respiratory tract disease become apparent after an incubation period of 2 to 5 days. The fauces are most commonly involved; however, disease may also occur at other sites, including the anterior nares, larynx, and tracheobronchial tree.

Anterior Nasal

Anterior nasal diphtheria is characterized by a mucopurulent discharge that may be slightly bloody. In more severe cases a white membrane develops on the anterior nasal mucosae and septum. Rarely, the membrane erodes through the nares and upper lip.[50] Systemic toxicity in anterior nasal diphtheria is uncommon, even in the presence of a pseudomembrane.[6]

Faucial

Early symptoms of infection of the tonsillar pillars and pharynx include sore throat, malaise, and low-grade fever (usually less than 39°C). Approximately 3 days later a pseudomembrane forms on the tonsils or proximal pharynx in 50% to 80% of individuals (Fig. 204.3).[52,53] The membrane is initially white, then becomes grayish-green or black and may extend to the soft palate, nasopharynx, laryngopharynx, or bronchi. Forceful removal of the membrane causes bleeding of the underlying mucosae. Approximately one-third of affected individuals develop a "bull neck" appearance as a result of cervical lymph node enlargement and submandibular edema.[54] Local complications of pharyngeal diphtheria include stridor, airway obstruction, and subsequent respiratory failure. The case fatality rate of pharyngeal diphtheria is approximately 10%. In the absence of a pseudomembrane, disease is less severe and associated with improved outcomes.[52,53]

Laryngeal and Tracheobronchial

Although primary infection of the larynx, trachea, and bronchial tree may occur, these sites are more often secondarily involved as a result

FIG. 204.3 Pharynx of a 39-year-old woman with bacteriologically confirmed diphtheria. Photograph taken 4 days after the onset of fever, malaise, and sore throat. Hemorrhage due to removal of the membrane by swabbing appears as dark area on the left.

FIG. 204.4 A diphtheria skin lesion on the leg. *(From Centers for Disease Control.* Public Health Image Library. Diphtheria Photos: Photo ID #1941. https://www.cdc.gov/diphtheria/about/photos.html.*)*

of pseudomembranous extension from the pharynx. Prominent symptoms include stridor, hoarseness, and a barking cough. Airway edema or membrane dislodgement leads to eventual respiratory embarrassment and asphyxiation.[6,50]

Cardiac Toxicity

Studies from Vietnam and former Soviet Union republics indicate that up to 25% of individuals with pharyngeal diphtheria develop cardiac toxicity.[53–56] Fever, tonsillar pseudomembrane, and "bull neck" appearance are predictive of cardiac involvement, which may occur acutely or approximately 10 days after the initial onset of symptoms. Electrocardiographic abnormalities in diphtheritic cardiomyopathy consist of ST-segment and T-wave changes and QT interval prolongation. Severe complications of cardiac involvement include cardiac dilatation, arrhythmias, and heart block. Approximately one-third of patients with diphtheritic cardiomyopathy suffer a fatal outcome; in Vietnam, third-degree atrioventricular block and ST-segment depressions/T-wave inversions were associated with worse outcomes. Resolution of electrocardiographic abnormalities occurred in all survivors.[56]

Neurologic Toxicity

Although less common than cardiac toxicity, neurologic complications of pharyngeal diphtheria are a cause of significant morbidity.[50,52–54] A local motor neuropathy, manifesting as paralysis of the soft palate and posterior pharyngeal wall, occurs initially. Afterward, bulbar and oculomotor neuropathies may develop, leading to further paralysis of the pharynx and involvement of the extraocular and ciliary muscles. Peripheral neuritis, occurring early in the disease or up to 3 months after respiratory symptoms have abated, is characterized by a descending motor neuropathy involving the diaphragm and limbs.[57–59] Cerebrospinal fluid analysis usually reveals a cytoalbuminologic dissociation, similar to Guillain-Barré syndrome; the latter may be distinguished from diphtheritic polyneuropathy by its characteristic ascending paralysis. Sensory involvement occurs in a stocking-and-glove distribution. Signs of autonomic dysfunction, such as hypotension and urinary retention, may also develop. Cranial nerve neuropathies tend to improve at around the same time during the disease course as peripheral nerve function worsens.[59] After several weeks of neurologic involvement, complete recovery of peripheral motor and sensory nerve function is the norm.

Other Complications

Acute kidney injury, due to direct activity of the exotoxin, may occur. The exotoxin has been shown to induce necrosis of the kidneys, liver, and adrenal glands in animal models.[60,61]

Cutaneous Diphtheria

Although widespread vaccination has led to a decline in the incidence of respiratory tract disease, cutaneous diphtheria has become increasingly recognized. Cutaneous infection due to toxigenic *C. diphtheriae* may occur in unimmunized individuals, but the majority of cases are caused by nontoxigenic strains. Outbreaks have been described in impoverished communities, among racial minorities, and in settings with high rates of alcoholism and IV drug use.[21,22,62] Cutaneous infection is well described in resource-limited settings, where asymptomatic skin carriage induces natural immunity in the host and also serves as an important reservoir for transmission to susceptible individuals in the community. Cutaneous transmission may be more efficient than the respiratory route and may lead to contamination of fomites, thereby facilitating reinfections during outbreaks. Cutaneous diphtheria classically manifests as an ulcerative lesion that may be associated with a pseudomembrane (Fig. 204.4). Skin involvement may present uncharacteristically, however, as a scaly, impetiginous, or erythematous lesion.[21] Coinfections with other organisms, including *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Arcanobacterium haemolyticum* have been described.[62]

Invasive Disease

Nontoxigenic *C. diphtheriae* strains are known to cause bacteremia with metastatic complications, including endocarditis, septic arthritis, pseudoaneurysms, and mycotic cerebral aneurysms.[37,63,64] Most cases of invasive disease have occurred in impoverished communities in individuals with comorbid conditions, such as diabetes mellitus, alcoholism, and IV drug use. The clinical presentation is often fulminant and may be associated with a fatal outcome.

Other Sites

Localized infection may occur at other sites. Cases of diphtheritic conjunctivitis, corneal ulcers, otitis media, mastoiditis, and vulvovaginitis have been described in the literature.[65,66]

DIAGNOSIS

Respiratory diphtheria should be considered in the differential diagnosis whenever an individual with appropriate epidemiologic risk factors (i.e., travel or residence within an epidemic or endemic setting; history of inadequate vaccination) presents with sore throat, low-grade fever, and cervical adenopathy. Other conditions that may have similar findings include: Streptococcal pharyngitis, infectious mononucleosis, epiglottitis, Vincent angina, retropharyngeal space infection, oropharyngeal candidiasis, and acute retroviral syndrome after human immunodeficiency virus (HIV) infection. Although streptococcal pharyngitis may present with tonsillar exudates, the infection is more acute in onset than diphtheria and is characterized by high fever and an exquisitely sore throat. Infectious mononucleosis tends to occur in adolescents and young adults, does not evolve into a toxic illness, and may be differentiated on the basis of positive serum heterophile antibodies or Epstein-Barr virus serologies. Epiglottitis is characterized by stridor and a barking cough; however, it is not associated with pharyngitis or a pseudomembrane. Vincent angina, or acute necrotizing ulcerative gingivitis, will often present as a gingival exudate that may extend to the pharynx in

an individual with fetid breath, poor dentition, and underlying gingival disease. The neck swelling associated with a retropharyngeal space infection may resemble the "bull neck" appearance of pharyngeal diphtheria. Retropharyngeal space infections do not have an associated pseudomembrane, though, and are readily diagnosed with an imaging study of the neck. Oropharyngeal candidiasis may present with tonsillar exudates and odynophagia if the esophagus is involved, but the infection is not characterized by fever and occurs in individuals with appropriate risk factors (e.g., cellular deficiency; use of inhaled corticosteroids). The acute retroviral syndrome seen in early HIV infection may be associated with pharyngitis, cervical adenopathy, and low-grade fever. Acute HIV infection does not evolve into a toxic illness characterized by stridor and impending respiratory failure, and it may be distinguished from diphtheria on the basis of epidemiologic risk factors (e.g., sexual history) and appropriate diagnostic testing.

Droplet or contact precautions should be instituted once respiratory or cutaneous diphtheria is suspected, respectively. Cultures should be performed on swabs of the throat, tonsils, nasopharynx, and any skin lesions. A carefully removed portion of the pseudomembrane and a swab of the underlying mucosae should also be cultured, if feasible. It is necessary to inform the microbiology laboratory of the suspicion of diphtheria such that selective media are used; *C. diphtheriae* may be discounted as a contaminant if specimens are only plated on blood agar. Specimens should be plated on both sheep blood agar to detect *Streptococcus* spp. and on selective tellurite-containing media, such as modified Loeffler or Tinsdale.[8,14] Potassium tellurite inhibits the growth of upper respiratory flora, and *Corynebacterium* spp. appear as black colonies with a brown halo. The organisms are club-shaped, gram-positive bacilli and may demonstrate "Chinese character" arrangement. Biochemical assays (e.g., API Coryne) differentiate the four biovars and *C. ulcerans*. *C. ulcerans* may cause an illness similar to diphtheria and may also elaborate diphtheria toxin; however, it is usually associated with zoonotic transmission and is urease positive, unlike *C. diphtheriae*.[67,68]

Guinea pigs were used for the detection of diphtheria toxin in clinical specimens until the middle of the 20th century, at which time the Elek immunodiffusion test supplanted in vivo testing. Due to inconsistencies and laboratory variation in interpretation of the Elek test, PCR of the *tox* gene was developed. An important limitation of *tox* PCR testing is that some *C. diphtheriae* strains may harbor the gene without expressing it. EIA, using monoclonal antibody to fragment A of the exotoxin, is highly accurate and able to overcome the drawbacks of both the Elek test and PCR.[69]

THERAPY

The mainstay of treatment of respiratory diphtheria is antitoxin therapy. Equine serum containing diphtheria antitoxin (DAT) was shown in 1898 to reduce mortality from 12% to 3% in what may have been the first randomized clinical trial.[70] DAT neutralizes circulating unbound toxin and is of greatest benefit in preventing disease progression when administered within the first 48 hours of symptoms.[6] To minimize the risk of serum sickness or anaphylaxis, the scratch test should be performed with a 1:1000 dilution of DAT in patients without a history of hypersensitivity to equine serum and, if negative, followed by an intradermal test with the same dilution. If there is a history of hypersensitivity or if either test is positive, desensitization to antitoxin should be performed.[71] DAT is administered intravenously at a dose of 20,000 to 40,000 U for pharyngeal or laryngeal disease of 2 days' duration or less; 40,000 to 60,000 U for nasopharyngeal disease; and 80,000 to 120,000 U for extensive disease with neck swelling or for disease greater than 3 days' duration.[71,72] Although the intravenous route is preferred for administration of DAT, intramuscular injection may be considered for less severe cases. There is currently a worldwide shortage of antitoxin due to decreased production over the past several years. In the United States DAT is only available under an Investigational New Drug (IND) protocol and can be obtained by contacting the Centers for Disease Control and Prevention (CDC) at 770-488-7100. Much higher antitoxin titers are needed for treatment compared with prevention of infection. Human donor serum does not contain a sufficient concentration of antitoxin for treatment; even with enrichment techniques, prohibitively high volumes of donor serum would be necessary to achieve such a concentration.[73] Efforts are currently underway to develop recombinant antibodies to exotoxin for treatment.[74]

Antibiotic therapy prevents the propagation of organisms and further elaboration of toxin while decreasing transmission. *C. diphtheriae* is susceptible to a wide range of antimicrobials, including β-lactams, erythromycin, ciprofloxacin, tetracycline, chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole, and rifampin. Penicillin and erythromycin have traditionally been used for therapy, although there have been concerns with erythromycin due to its arrhythmogenic potential and gastrointestinal side effects. Moreover, despite erythromycin's greater in vitro activity than penicillin, there have been reports of strains that are macrolide resistant.[75–77] A randomized trial comparing the two antibiotics in 44 Vietnamese children showed that defervescence occurred more quickly with penicillin; however, there was no difference between the two arms in time to bacteriologic clearance or resolution of pseudomembrane.[75] CDC guidelines recommend that treatment consist of 14 days of oral or parenteral erythromycin at 40 mg/kg/day (maximum 2 g/day) or intramuscular procaine penicillin G at a dose of 300,000 U twice daily for those weighing 10 kg or less or 600,000 U twice daily for those weighing more than 10 kg.[6] Procaine penicillin G may be switched to penicillin VK once oral intake is feasible.

Airway management is critical during treatment of respiratory diphtheria, as respiratory failure may occur as a result of extension of the pseudomembrane, leading to obstruction or aspiration. Preemptive endotracheal intubation is recommended in most instances, and severe cases may require tracheotomy.[52] Patients should be hospitalized in units with cardiac monitoring capabilities for early diagnosis and management of myocardial toxicity. Droplet precautions should be maintained until the 14-day course of antibiotic treatment has been completed and two cultures taken 24 hours apart after the discontinuation of antibiotics are negative. Vaccination with diphtheria toxoid after convalescence is necessary, as natural infection may not induce an adequate level of protective immunity.

Cutaneous diphtheria is treated with the same antibiotic regimen as that for respiratory disease. DAT has not been shown to be beneficial in the treatment of cutaneous diphtheria, as the majority of cases are caused by nontoxigenic strains. There is limited data on treatment of invasive or systemic nontoxigenic infection. A case series on the treatment of *C. diphtheriae* endocarditis reported similar outcomes with combination therapy consisting of a β-lactam or vancomycin in conjunction with an aminoglycoside as with single-drug therapy.[78] A literature review of 29 cases of endocarditis by the same authors found no significant difference in mortality or in need for surgical treatment with combination therapy. The review was limited by its small sample size and heterogeneity, especially relating to the duration of aminoglycoside therapy. Given the paucity of data, complicated systemic infection, such as endocarditis or septic arthritis, should be treated for a prolonged course; combination therapy with an aminoglycoside for at least a short duration is not unreasonable if there are no contraindications precluding such therapy.

PREVENTION

Incorporation of the diphtheria toxoid vaccine into routine immunization schedules has resulted in a dramatic decrease in the global disease incidence. In adequately immunized individuals the vaccine has a clinical efficacy of approximately 97% in preventing the development of toxigenic disease.[6] The minimum concentration of antitoxin needed for protection is 0.01 IU antitoxin/mL. A level of 0.1 IU antitoxin/mL or greater, affording full protection against disease, is achieved after administration of a series of four primary doses in children or three in adults.[6,79,80] Waning immunity is of significant concern in regions with high rates of childhood vaccination but lack of booster coverage in older children and adults. A British study demonstrated that, in the absence of boosting, only 50% of adults older than 60 years were immune to diphtheria.[81]

Pediatric formulations of the vaccine contain 15 to 25 Lf ("limes flocculationis" [limit of flocculation]) of diphtheria toxoid. Local and systemic (e.g., anaphylaxis, urticaria, angioedema) reactions are common in adults and older children who are vaccinated with this dose.[80,82] Adult formulations of toxoid vaccine, administered to those age 7 years and older, contain less toxoid (2–2.5 Lf), are less likely to cause reactions,

and are as equally immunogenic as the children's dose. Diphtheria toxoid is combined with tetanus toxoid in both pediatric and reduced-dose adult formulations of the vaccine (DT and Td, respectively); both formulations are available as combined vaccines with acellular pertussis components (DTaP and Tdap, respectively). DTaP vaccines are also available in combination with vaccines against hepatitis B virus, poliovirus (inactivated vaccine), and *Haemophilus influenzae* serotype b.

The following is a summary of the schedule for vaccination against diphtheria, as recommended by the Advisory Committee on Immunization Practices and the CDC[6,83,84]:

From 6 weeks to 7 years of age: A series of three doses of DTaP spaced 4 to 8 weeks apart should be started at age 2 months and no earlier than 6 weeks of age. After a minimum interval of 6 months after the third dose, a fourth primary dose is given at age 15 to 18 months. A fifth dose is administered at age 4 to 6 years (around the time of school entry) if the fourth dose was administered before the fourth birthday. The series does not need to be restarted if a dose is missed; in this case the next dose should be administered and the series resumed.

For unvaccinated individuals 7 years of age and older: A primary series of three doses of Td is administered with an interval of at least 4 weeks between the first two doses and 6 to 12 months between the second and third doses. The first vaccination in the series should ideally be with Tdap.

For individuals 10 years of age and older who have completed the primary vaccination series: A booster dose of Td should be given every 10 years. The first booster should be with Tdap if it was never administered previously. Pregnant women should receive a dose of Tdap at 27 to 36 weeks' gestation of each pregnancy, preferably during the earlier weeks of this interval, regardless of their vaccination history.

Vaccination does not prevent infection, transmission, or asymptomatic carriage of *C. diphtheriae*. Close contacts should be vaccinated with a booster dose if more than 5 years have elapsed since their most recent vaccination. If their vaccination history is unknown or incomplete, a primary series should be initiated or completed. All contacts should also be treated with a single dose of intramuscular benzathine penicillin G (600,000 U if <6 years old; 1.2 million U if 6 years or older) or 7 to 10 days of erythromycin (40 mg/kg/d for children; 1 g/d for adults).[6] Although increasingly rare, any identified carriers of toxigenic strains should be treated with a similar course of benzathine penicillin G or erythromycin. Droplet precautions should be maintained until two cultures obtained at least 24 hours apart and no earlier than 14 days after the completion of antibiotic therapy are negative. If cultures remain positive, erythromycin should be administered for another 10 days, due to its greater effectiveness in eradicating the carrier state, and follow-up cultures obtained after the completion of therapy.[85,86]

Key References

The complete reference list is available online at Expert Consult.

6. Centers for Disease Control and Prevention. Diphtheria. In: Hamborsky J, Kroger A, Wolfe S, eds. *Epidemiology and Prevention of Vaccine-Preventable Diseases*. 13th ed. Washington D.C.: Public Health Foundation; 2015:107–118.
7. Vitek CR, Wharton M. Diphtheria in the former Soviet Union: reemergence of a pandemic disease. *Emerg Infect Dis*. 1998;4:539–550.
8. Murphy JR. *Corynebacterium diphtheriae*. In: Baron S, ed. *Medical Microbiology*. 4th ed. Galveston, TX: University of Texas Medical Branch at Galveston; 1996:413–422.
14. Efstratiou J, Engler KH, Mazurova IK, et al. Current approaches to the laboratory diagnosis of diphtheria. *J Infect Dis*. 2000;181:S138–S145.
20. Galazka AM, Roberson SE. Diphtheria: changing patterns in the developing world and the industrialized world. *Eur J Epidemiol*. 1995;11:107–117.
21. Belsey MA, Sinclair M, Roder MR, et al. *Corynebacterium diphtheriae* skin infections in Alabama and Louisiana: a factor in the epidemiology of diphtheria. *N Engl J Med*. 1969;280:135–141.
22. Pederson AH, Spearman J, Tronca E, et al. Diphtheria on Skid Road, Seattle, Wash., 1972-75. *Public Health Rep*. 1977;92:336–342.
23. Galazka MA, Roberson SE, Oblapenko GP. Resurgence of diphtheria. *Eur J Epidem*. 1995;11:95–105.
25. Vitek CR, Wharton M. Diphtheria in the former Soviet Union: reemergence of a pandemic disease. *Emerg Infect Dis*. 1998;4:539–550.
27. Brooks GF, Bennett JV, Feldman RA. Diphtheria in the United States, 1959-1970. *J Infect Dis*. 1974;129:172–178.
28. Chen RT, Broome CV, Weinstein RA, et al. Diphtheria in the United States, 1971-81. *Am J Public Health*. 1985;75:1393–1397.
29. World Health Organization. Diphtheria Vaccine: WHO Position Paper: August 2017. *Wkly Epidemiol Rec*. 2017;92:417–435. http://www.who.int/immunization/policy/position_papers/wer_31_diphtheria_updated_position_paper.pdf?ua=1. Accessed December 2017.
30. Clarke KEN. Review of the Epidemiology of Diphtheria 2000-2016. US Centers for Disease Control and Prevention. http://www.who.int/immunization/sage/meetings/2017/april/1_Final_report_Clarke_april3.pdf. Accessed December 2017.
32. Galazka A, Dittman S. The changing epidemiology of diphtheria in the vaccine era. *J Infect Dis*. 2000;181:S2–S9.
35. Reacher M, Ramsay M, White J, et al. Nontoxigenic *Corynebacterium diphtheriae*: an emerging pathogen in England and Wales? *Emerg Infect Dis*. 2000;6:640–645.
37. Gubler J, Huber-Schneider C, Gruner E, et al. An outbreak of nontoxigenic *Corynebacterium diphtheriae* infection: single bacterial clone causing invasive infection among Swiss drug users. *Clin Infect Dis*. 1998;27:1295–1298.
50. Hadfield TL, McEvoy P, Polotsky Y, et al. The pathology of diphtheria. *J Infect Dis*. 2000;181(suppl 1):S116–S120.
52. Dobie RA, Tobey DN. Clinical features of diphtheria in the respiratory tract. *JAMA*. 1979;242:2197–2201.
53. Quick ML, Sutter RW, Kobaidze K, et al. Epidemic diphtheria in the Republic of Georgia, 1993-1996; risk factors for fatal outcome among hospitalized patients. *J Infect Dis*. 2000;181(suppl 1):S130–S137.
54. Khadirova R, Kartoglu HU, Strebel PM. Clinical characteristics and management of 676 hospitalized diphtheria cases, Kyrgyz Republic, 1995. *J Infect Dis*. 2000;181(suppl 1):S110–S115.
55. Lumio JT, Groundstroem KW, Melnick OB, et al. Electrocardiographic abnormalities in patients with diphtheria: a prospective study. *Am J Med*. 2004;116:78–83.
56. Kneen R, Nguyn MD, Solomon T, et al. Clinical features and predictors of diphtheritic cardiomyopathy in Vietnamese children. *Clin Infect Dis*. 2004;39:1591–1598.
57. Manikyamba D, Satyavani A, Deepa P. Diphtheritic polyneuropathy in the wake of resurgence of diphtheria. *J Pediatr Neurosci*. 2015;10:331–334.
59. Piradov MA, Pirogov VN, Popova LM, et al. Diphtheritic polyneuropathy: clinical analysis of severe forms. *Arch Neurol*. 2001;58:1438–1442.
61. Holmes RK. Biology and molecular epidemiology of diphtheria toxin and the *tox* gene. *J Infect Dis*. 2000;181(suppl 1):S156–S167.
62. Lowe CF, Bernard KA, Romney MG. Cutaneous diphtheria in the urban poor population of Vancouver, British Columbia, Canada: a 10-year review. *J Clin Microbiol*. 2011;49:2664–2666.
63. Holthouse DJ, Power B, Kermode A, et al. Non-toxigenic *Corynebacterium diphtheriae*: two cases and review of the literature. *J Infect*. 1998;37:62–66.
64. Romney MG, Roscoe DL, Bernard K, et al. Emergence of an invasive clone of nontoxigenic *Corynebacterium diphtheriae* in the urban poor population of Vancouver, Canada. *J Clin Microbiol*. 2006;44:1625–1629.
67. Sangal V, Nieminen L, Weinhardt B, et al. Diphtheria-like disease caused by toxigenic *Corynebacterium ulcerans* strain. *Emerg Infect Dis*. 2014;20:1257–1258.
69. Engler KH, Efstratiou A. Rapid enzyme immunoassay for determination of toxigenicity among clinical isolates of corynebacteria. *J Clin Microbiol*. 2000;38:1385–1389.
70. Hróbjartsson A, Gøtzsche PC, Gluud C. The controlled trial turns 100 years: Fibiger's trial of serum treatment of diphtheria. *BMJ*. 1998;317:1243–1245.
71. Stiehm ER, Keller MA. Passive immunization. In: Plotkin SA, Orenstein WA, Offit PA, eds. *Vaccines*. 6th ed. Philadelphia: Saunders; 2013:80–87.
72. Centers for Disease Control and Prevention. Expanded access investigational new drug (IND) protocol: Use of diphtheria antitoxin (DAT) for suspected diphtheria cases. Protocol CDC IRB #4167. Version 7.0; September 21, 2016. https://www.cdc.gov/diphtheria/downloads/protocol.pdf.
74. Huygen K. Development of human monoclonal antibodies to diphtheria toxin: a solution for the increasing lack of equine DAT for therapeutic use? *Virulence*. 2016;7:613–615.
75. Kneen R, Pham NG, Solomon T, et al. Penicillin vs. erythromycin in the treatment of diphtheria. *Clin Infect Dis*. 1998;27:845–850.
77. Zamiri I, McEntegart MG. The sensitivity of diphtheria bacilli to eight antibiotics. *J Clin Path*. 1972;25:716–717.
78. Muttaiyah S, Best EJ, Freeman JT, et al. *Corynebacterium diphtheriae* endocarditis: a case series and review of the treatment approach. *Int J Infect Dis*. 2011;15:584–588.
79. Brennan M, Vitek C, Strebel P, et al. How many doses of diphtheria toxoid are required for protection in adults? Results of a case-control study among 40- to 49-year-old adults in the Russian Federation. *J Infect Dis*. 2000;181(suppl 1):S193–S196.
80. Galazka AM, Roberson SE. Immunization against diphtheria with special emphasis on immunization of adults. *Vaccine*. 1996;14:845–857.
82. Myers MG, Beckman CW, Vosdingh RA, et al. Primary immunization with tetanus and diphtheria toxoids. Reaction rates and immunogenicity in older children and adults. *JAMA*. 1982;248:2478–2480.
83. Robinson CL, Romero JR, Kempe A, et al. Advisory Committee on Immunization Practices recommended immunization schedule for children and adolescents aged 18 years or younger — United States, 2017. *MMWR Morb Mortal Wkly Rep*. 2017;66:134–135.
84. Kim DK, Riley LE, Harriman KH, et al. Advisory Committee on Immunization Practices recommended immunization schedule for adults aged 19 years or older—United States, 2017. *MMWR Morb Mortal Wkly Rep*. 2017;66:136–138.
85. McCloskey RV, Green MJ, Eller J, et al. Treatment of diphtheria carriers: benzathine penicillin, erythromycin and clindamycin. *Ann Intern Med*. 1974;81:788–791.
86. Miller LW, Bickham S, Jones WL, et al. Diphtheria carriers and the effect of erythromycin therapy. *Antimicrob Agents Chemother*. 1974;6:166–169.

References

1. Shulman ST. The history of pediatric infectious diseases. *Pediatr Res*. 2004;55:163–176.
2. Caulfield E. A history of the terrible epidemic, vulgarly called the throat distemper, as it occurred in his majesty's New England colonies between 1735 and 1740. *Yale J Biol Med*. 1939;11:219–272.
3. English PC. Diphtheria and theories of infectious disease: centennial appreciation of the critical role of diphtheria in the history of medicine. *Pediatrics*. 1985;76:1–9.
4. Hajj Hussein I, Chams N, Chams S, et al. Vaccines through centuries: major cornerstones of global health. *Front Public Health*. 2015;3:1–16.
5. Roush SW, Murphy TV, Vaccine-Preventable Disease Table Working Group. Historical comparisons of morbidity and mortality for vaccine-preventable disease in the United States. *JAMA*. 2007;298:2155–2163.
6. Centers for Disease Control and Prevention. Diphtheria. In: Hamborsky J, Kroger A, Wolfe S, eds. *Epidemiology and Prevention of Vaccine-Preventable Diseases*. 13th ed. Washington D.C.: Public Health Foundation; 2015: 107–118.
7. Vitek CR, Wharton M. Diphtheria in the former Soviet Union: reemergence of a pandemic disease. *Emerg Infect Dis*. 1998;4:539–550.
8. Murphy JR. *Corynebacterium diphtheriae*. In: Baron S, ed. *Medical Microbiology*. 4th ed. Galveston, TX: University of Texas Medical Branch at Galveston; 1996:413–422.
9. Whitley OR, Damon SR. A transparent dextrose serum tellurite plating medium; its use as an adjunct to microscopic examination of smears made from Loeffler slants in routine diphtheria diagnosis. *Public Health Rep*. 1949;64:201–212.
10. Soto A, Zapardiel J, Soriano F. Evaluation of API Coryne system for identifying coryneform bacteria. *J Clin Pathol*. 1994;47:756–759.
11. Funke G, von Graevenitz A, Clarridge JE 3rd, et al. Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev*. 1997;10:125–129.
12. Mokrousev I. Resolution threshold of current molecular epidemiology of diphtheria. *Emerg Infect Dis*. 2014;20: 1937–1938.
13. Bolt F, Cassiday P, Tondella ML, et al. Multilocus sequence typing identifies evidence for recombination and two distinct lineages of *Corynebacterium diphtheriae*. *J Clin Microbiol*. 2010;48:4177–4185.
14. Efstratiou J, Engler KH, Mazurova IK, et al. Current approaches to the laboratory diagnosis of diphtheria. *J Infect Dis*. 2000;181:S138–S145.
15. Engler KH, Efstratiou J, Norn D, et al. Immunochromatographic strip test for rapid detection of diphtheria toxin: description and multicenter evaluation in areas of low and high prevalence of diphtheria. *J Clin Microbiol*. 2002;40:80–83.
16. Peck H. The transmission of diphtheria by non-sufferers. *Br Med J*. 1895;1:971.
17. Henricson B, Segarra M, Garvin J, et al. Toxigenic *Corynebacterium diphtheriae* associated with an equine wound infection. *J Vet Diagn Invest*. 2000;12:253–257.
18. Corboz L, Thoma R, Braun U, et al. Isolation of *Corynebacterium diphtheriae* subsp. *belfanti* from a cow with chronic active dermatitis [in German]. *Schweiz Arch Tierheilkd*. 1996;138:596–599.
19. Hall AJ, Cassiday PK, Bernard KA, et al. Novel *Corynebacterium diphtheriae* in domestic cats. *Emerg Infect Dis*. 2010;16:688–691.
20. Galazka AM, Roberson SE. Diphtheria: changing patterns in the developing world and the industrialized world. *Eur J Epidemiol*. 1995;11:107–117.
21. Belsey MA, Sinclair M, Roder MR, et al. *Corynebacterium diphtheriae* skin infections in Alabama and Louisiana: a factor in the epidemiology of diphtheria. *N Engl J Med*. 1969;280:135–141.
22. Pederson AH, Spearman J, Tronca E, et al. Diphtheria on Skid Road, Seattle, Wash., 1972-75. *Public Health Rep*. 1977;92:336–342.
23. Galazka MA, Roberson SE, Oblapenko GP. Resurgence of diphtheria. *Eur J Epidem*. 1995;11:95–105.
24. Frost WH, Frobisher M, van Volkenburgh VA, et al. Diphtheria in Baltimore: a comparative study of morbidity, carrier prevalence and antitoxin immunity in 1921-1924 and 1933-1936. *Am J Hyg*. 1936;24:568–586.
25. Vitek CR, Wharton M. Diphtheria in the former Soviet Union: reemergence of a pandemic disease. *Emerg Infect Dis*. 1998;4:539–550.
26. Van Panhuis WG, Grefenstette J, Jung SY, et al. Contagious diseases in the United States from 1888 to the present. *N Engl J Med*. 2013;369:2152–2158.
27. Brooks GF, Bennett JV, Feldman RA. Diphtheria in the United States, 1959-1970. *J Infect Dis*. 1974;129:172–178.
28. Chen RT, Broome CV, Weinstein RA, et al. Diphtheria in the United States, 1971-81. *Am J Public Health*. 1985;75:1393–1397.
29. World Health Organization. Diphtheria Vaccine: WHO Position Paper: August 2017. Diphtheria Vaccine: WHO Position Paper: August 2017. *Wkly Epidemiol Rec*. 2017;92:417–435. http://www.who.int/immunization/policy/position_papers/wer_31_diphtheria_updated_position_paper.pdf?ua=1. Accessed December 2017.
30. Clarke KEN. Review of the epidemiology of diphtheria 2000-2016. US Centers for Disease Control and Prevention. http://www.who.int/immunization/sage/meetings/2017/april/1_Final_report_Clarke_april3.pdf. Accessed December 2017.
31. Sangal L, Joshi S, Anandan S, et al. Resurgence of diphtheria in North Kerala, India, 2016: laboratory supported case-based surveillance outcomes. *Front Public Health*. 2017;5:218.
32. Galazka A, Dittman S. The changing epidemiology of diphtheria in the vaccine era. *J Infect Dis*. 2000;181:S2–S9.
33. Khuri-Bulos N, Hamzah Y, Sammerrai SM, et al. The changing epidemiology of diphtheria in Jordan. *Bull World Health Organ*. 1988;66:65–68.
34. Loevinsohn BP. The changing age structure of diphtheria patients: evidence for the effectiveness of EPI in the Sudan. *Bull World Health Organ*. 1990;68:353–357.
35. Reacher M, Ramsay M, White J, et al. Nontoxigenic *Corynebacterium diphtheriae:* an emerging pathogen in England and Wales? *Emerg Infect Dis*. 2000;6:640–645.
36. Zasada AA. Nontoxigenic highly pathogenic clone of *Corynebacterium diphtheriae,* Poland, 2004-2012. *Emerg Infect Dis*. 2013;19:1870–1872.
37. Gubler J, Huber-Schneider C, Gruner E, et al. An outbreak of nontoxigenic *Corynebacterium diphtheriae* infection: single bacterial clone causing invasive infection among Swiss drug users. *Clin Infect Dis*. 1998;27:1295–1298.
38. Zakikhany K, Neal S, Efstratiou A. Emergence and molecular characterization of non-toxigenic *tox* gene-bearing *Corynebacterium diphtheriae* biovar *mitis* in the United Kingdom, 2003-2012. *Euro Surveill*. 2013;19:pii:20819.
39. Mattos-Guaraldi AL, Formiga LC, Andrade AF. Trans-sialidase activity for sialic acid incorporation on *Corynebacterium diphtheriae*. *FEMS Microbiol Lett*. 1998;168:167–172.
40. Broadway MM, Rogers EA, Chang C, et al. Pilus gene pool variation and the virulence of *Corynebacterium diphtheriae* clinical isolates during infection of a nematode. *J Bacteriol*. 2013;195:3774–3783.
41. Mandlik A, Swierczynski A, Ton-That H. *Corynebacterium diphtheriae* employs specific minor pilins to target human pharyngeal epithelial cells. *Mol Microbiol*. 2007;64:111–124.
42. Antunes CA, Sanches dos Santos L, Hacker E, et al. Characterization of DIP0733, a multi-functional virulence factor of *Corynebacterium diphtheriae*. *Microbiology*. 2015;161:639–647.
43. Peixoto RS, Antunes CA, Louredo LS, et al. Functional characterization of the collagen-binding protein DIP2093 and its influence on host-pathogen interaction and arthritogenic potential of *Corynebacterium diphtheriae*. *Microbiology*. 2017;163:692–701.
44. Gomes DL, Martins CA, Faria LM, et al. *Corynebacterium diphtheriae* as an emerging pathogen in nephrostomy catheter-related infection: evaluation of traits associated with bacterial virulence. *J Med Microbiol*. 2009;58:1419–1427.
45. Peixoto RS, Hacker E, Antunes CA, et al. Pathogenic properties of a *Corynebacterium diphtheriae* strain isolated from a case of osteomyelitis. *J Med Microbiol*. 2016;65:1311–1321.
46. Sabbadini PS, Genovez MR, Silva CF, et al. Fibrinogen binds to nontoxigenic and toxigenic *Corynebacterium diphtheriae* strains. *Mem Inst Oswaldo Cruz*. 2010;105:706–711.
47. Kaul G, Pattan G, Rafeequi T. Eukaryotic elongation factor-2 (eEF2): its regulation and peptide chain elongation. *Cell Biochem Funct*. 2011;29:227–234.
48. Yamaizumi M, Mekada E, Uchida T, et al. One molecule of diphtheria toxin fragment A introduced into a cell can kill the cell. *Cell*. 1978;15:245–250.
49. Gill DM. Bacterial toxins: a table of lethal amounts. *Microbiol Rev*. 1982;46:86–94.
50. Hadfield TL, McEvoy P, Polotsky Y, et al. The pathology of diphtheria. *J Infect Dis*. 2000;181(suppl 1):S116–S120.
51. Byard RW. Diphtheria—"the strangling angel" of children. *J Forensic Leg Med*. 2013;20:65–68.
52. Dobie RA, Tobey DN. Clinical features of diphtheria in the respiratory tract. *JAMA*. 1979;242:2197–2201.
53. Quick ML, Sutter RW, Kobaidze K, et al. Epidemic diphtheria in the Republic of Georgia, 1993-1996; risk factors for fatal outcome among hospitalized patients. *J Infect Dis*. 2000;181(suppl 1):S130–S137.
54. Khadirova R, Kartoglu HU, Strebel PM. Clinical characteristics and management of 676 hospitalized diphtheria cases, Kyrgyz Republic, 1995. *J Infect Dis*. 2000;181(suppl 1):S110–S115.
55. Lumio JT, Groundstroem KW, Melnick OB, et al. Electrocardiographic abnormalities in patients with diphtheria: a prospective study. *Am J Med*. 2004;116: 78–83.
56. Kneen R, Nguyn MD, Solomon T, et al. Clinical features and predictors of diphtheritic cardiomyopathy in Vietnamese children. *Clin Infect Dis*. 2004;39:1591–1598.
57. Manikyamba D, Satyavani A, Deepa P. Diphtheritic polyneuropathy in the wake of resurgence of diphtheria. *J Pediatr Neurosci*. 2015;10:331–334.
58. Alesen LA. Postdiphtheritic paralysis of the diaphragm. *JAMA*. 1925;84:730–731.
59. Piradov MA, Pirogov VN, Popova LM, et al. Diphtheritic polyneuropathy: clinical analysis of severe forms. *Arch Neurol*. 2001;58:1438–1442.
60. Jayashree M, Shruthi N, Singhi S. Predictors of outcome in patients with diphtheria receiving intensive care. *Indian Pediatr*. 2006;43:155–160.
61. Holmes RK. Biology and molecular epidemiology of diphtheria toxin and the *tox* gene. *J Infect Dis*. 2000;181(suppl 1):S156–S167.
62. Lowe CF, Bernard KA, Romney MG. Cutaneous diphtheria in the urban poor population of Vancouver, British Columbia, Canada: a 10-year review. *J Clin Microbiol*. 2011;49:2664–2666.
63. Holthouse DJ, Power B, Kermode A, et al. Non-toxigenic *Corynebacterium diphtheriae*: two cases and review of the literature. *J Infect*. 1998;37:62–66.
64. Romney MG, Roscoe DL, Bernard K, et al. Emergence of an invasive clone of nontoxigenic *Corynebacterium diphtheriae* in the urban poor population of Vancouver, Canada. *J Clin Microbiol*. 2006;44:1625–1629.
65. Chandler JW, Milam F. Diphtheria corneal ulcers. *Arch Ophthalmol*. 1978;96:53–56.
66. Revathi G, Goyal A. Primary diphtheritic otitis media and mastoiditis. *Indian J Otolaryngol Head Neck Surg*. 1998;50:178–180.
67. Sangal V, Nieminen L, Weinhardt B, et al. Diphtheria-like disease caused by toxigenic *Corynebacterium ulcerans* strain. *Emerg Infect Dis*. 2014;20:1257–1258.
68. Konrad R, Hormansdorfer S, Sing A. Possible human-to-human transmission of toxigenic *Corynebacterium ulcerans*. *Clin Microbiol Infect*. 2015;21:768–771.
69. Engler KH, Efstratiou A. Rapid enzyme immunoassay for determination of toxigenicity among clinical isolates of corynebacteria. *J Clin Microbiol*. 2000;38:1385–1389.
70. Hróbjartsson A, Gøtzsche PC, Gluud C. The controlled trial turns 100 years: Fibiger's trial of serum treatment of diphtheria. *BMJ*. 1998;317:1243–1245.
71. Stiehm ER, Keller MA. Passive immunization. In: Plotkin SA, Orenstein WA, Offit PA, eds. *Vaccines*. 6th ed. Philadelphia: Saunders; 2013:80–87.
72. Centers for Disease Control and Prevention. Expanded access investigational new drug (IND) protocol: Use of diphtheria antitoxin (DAT) for suspected diphtheria cases. Protocol CDC IRB #4167. Version 7.0; September 21, 2016. https://www.cdc.gov/diphtheria/downloads/protocol.pdf.
73. McCloskey RV, Smilack J. Diphtheria antitoxin content of human immune serum globulins. *Ann Intern Med*. 1972;77:757–758.
74. Huygen K. Development of human monoclonal antibodies to diphtheria toxin: a solution for the increasing lack of equine DAT for therapeutic use? *Virulence*. 2016;7: 613–615.
75. Kneen R, Pham NG, Solomon T, et al. Penicillin vs. erythromycin in the treatment of diphtheria. *Clin Infect Dis*. 1998;27:845–850.
76. Maple PA, Efstratiou A, Tseneva G, et al. The in-vitro susceptibilities of toxigenic strains of *Corynebacterium diphtheriae* isolated in northwestern Russia and surrounding areas to ten antibiotics. *J Antimicrob Chemother*. 1994;34:1037–1040.
77. Zamiri I, McEntegart MG. The sensitivity of diphtheria bacilli to eight antibiotics. *J Clin Path*. 1972;25:716–717.
78. Muttaiyah S, Best EJ, Freeman JT, et al. *Corynebacterium diphtheriae* endocarditis: a case series and review of the treatment approach. *Int J Infect Dis*. 2011;15:584–588.
79. Brennan M, Vitek C, Strebel P, et al. How many doses of diphtheria toxoid are required for protection in adults? Results of a case-control study among 40- to 49-year-old adults in the Russian Federation. *J Infect Dis*. 2000;181(suppl 1):S193–S196.
80. Galazka AM, Roberson SE. Immunization against diphtheria with special emphasis on immunization of adults. *Vaccine*. 1996;14:845–857.
81. Maple PA, Jones CS, Wall EC, et al. Immunity to diphtheria and tetanus in England and Wales. *Vaccine*. 2000;19:167–173.
82. Myers MG, Beckman CW, Vosdingh RA, et al. Primary immunization with tetanus and diphtheria toxoids. Reaction rates and immunogenicity in older children and adults. *JAMA*. 1982;248:2478–2480.

83. Robinson CL, Romero JR, Kempe A, et al. Advisory Committee on Immunization Practices recommended immunization schedule for children and adolescents aged 18 years or younger—United States, 2017. *MMWR Morb Mortal Wkly Rep*. 2017;66:134–135.
84. Kim DK, Riley LE, Harriman KH, et al. Advisory Committee on Immunization Practices recommended immunization schedule for adults aged 19 years or older—United States, 2017. *MMWR Morb Mortal Wkly Rep*. 2017;66:136–138.
85. McCloskey RV, Green MJ, Eller J, et al. Treatment of diphtheria carriers: benzathine penicillin, erythromycin and clindamycin. *Ann Intern Med*. 1974;81:788–791.
86. Miller LW, Bickham S, Jones WL, et al. Diphtheria carriers and the effect of erythromycin therapy. *Antimicrob Agents Chemother*. 1974;6:166–169.

205 Other Coryneform Bacteria, *Arcanobacterium haemolyticum*, and Rhodococci

Rose Kim and Annette C. Reboli

SHORT VIEW SUMMARY

Definition
- Coryneform bacteria encompass several genera, of which *Corynebacterium* is the most frequently encountered in clinical infections.
- Coryneform bacteria are characterized as irregularly shaped, non–spore-forming, aerobic, gram-positive rods.

Epidemiology
- Coryneform bacteria are ubiquitous in the environment (soil and water), commensal colonizers of skin and mucous membranes in humans, and commensals in animals.
- Infections caused by coryneform bacteria are broadly categorized as community acquired or nosocomial; sporadic cases of zoonoses have been reported.
- *Rhodococcus equi* usually occurs in individuals with defective cell-mediated immunity, particularly with human immunodeficiency virus infection, with or without a history of animal exposure.

Microbiology
- Coryneform bacteria readily grow on standard culture media. For lipophilic strains, growth is enhanced with addition of Tween 80.
- Species identification and antimicrobial testing of coryneform bacteria are recommended when specimens are collected from normally sterile sites, high colony counts are present with a strong leukocyte reaction, or high colony counts of *Corynebacterium urealyticum* are recovered from urine culture.
- Molecular tests, such as 16s ribosomal RNA sequencing, and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry are better than biochemical tests for species identification of coryneforms and *Rhodococcus*.

Diagnosis
- Coryneform bacteria are considered clinically significant when patients present with symptoms consistent with infection, along with recovery of bacteria as aforementioned (see "Microbiology").

Therapy
- Coryneform bacteria are uniformly susceptible to glycopeptides, such as vancomycin, and most strains are susceptible to daptomycin and linezolid.
- *R. equi* is usually susceptible to vancomycin, teicoplanin, erythromycin, fluoroquinolones, rifampin, carbapenems, aminoglycosides, and linezolid.

Prevention
- Prevention of infections caused by coryneform bacteria includes proper skin antisepsis before invasive procedures and caution when handling animals.

CORYNEFORM BACTERIA OTHER THAN *CORYNEBACTERIUM DIPHTHERIAE*

Corynebacterium was proposed as a genus by Lehmann and Neumann in 1896, who derived the name from the Greek *koryne,* which means "club," and *bacterion,* meaning "little rod."[1] The coryneforms are a diverse group of organisms. *Corynebacterium diphtheriae* serves as the type species, leading to the term *diphtheroids* to describe other bacteria sharing similar morphologic features. Also known as *coryneform bacteria,* bacteria demonstrating morphologic characteristics similar to those of corynebacteria include the genera *Corynebacterium, Arcanobacterium, Trueperella, Brevibacterium, Dermabacter, Microbacterium, Rothia, Turicella, Arthrobacter, Oerskovia, Leifsonia, Helcobacillus, Exiguobacterium, Cellulomonas, Cellulosimicrobium, Curtobacterium, Auritidibacter, Janibacter, Pseudoclavibacter, Brachybacterium,* and *Knoellia*.[2,3] The 16S ribosomal RNA (rRNA) sequencing data show that the genera *Corynebacterium* and *Turicella* are more related to the partially acid-fast bacteria and to the genus *Mycobacterium* than to the other coryneforms discussed in this chapter.[3]

Coryneform bacteria are widely distributed in the environment as normal inhabitants of soil and water. They are commensals colonizing the skin and mucous membranes of humans and other animals.[4,5] In the hospital setting, coryneform bacteria may be cultured from the hospital environment, including surfaces and medical equipment; corynebacteria are able to produce biofilm.[6] Coryneform bacteria other than *C. diphtheriae* have been isolated frequently in clinical specimens and were commonly considered contaminants without clinical significance. There is an increasing body of evidence of the pathogenicity of the coryneform bacteria, particularly as a cause of nosocomial infection in hospitalized and immunocompromised patients.[7,8] Several members of the genus *Corynebacterium* are better known as pathogens in animals and only incidentally cause infection in humans as zoonoses.

The coryneform bacteria are pleomorphic, demonstrating different forms at various stages of the life cycle, irregularly shaped gram-positive rods that are aerobically cultured, not spore forming, and not partially acid fast.[2,3] A history of misidentification of coryneform bacteria has made interpretation of the medical literature difficult. Initial identification is aided by observation of colony size and appearance, and the presence or absence of hemolysis on sheep blood agar. Odor production by colonies assists in identification, particularly of *Brevibacterium casei* and *Corynebacterium urealyticum*. Several of the medically relevant coryneform bacteria are lipophilic, demonstrating enhanced growth with the addition of Tween 80 to the culture medium.

True corynebacteria demonstrate club-shaped gram-positive rods on Gram staining, whereas other coryneform bacteria may not appear distinctly club shaped. Cells demonstrate variable sizes and appearance, from coccoid to bacillary forms, depending on the stage of the life cycle, and Gram-stain results may be uneven. Coryneform bacteria typically form arrangements such as "Chinese letters" or picket-fence configurations as a result of "snapping" after the cells divide. Lack of spore formation helps distinguish them from *Bacillus* species.[2]

The spectrum of human infections attributed to the coryneform bacteria is broad but can be understood in two general categories: community-acquired infections and nosocomial infections. Community-acquired infections include pharyngitis, skin and soft tissue infections, native valve endocarditis, genitourinary tract infections, acute and chronic prostatitis, and periodontal infections (Table 205.1).[9,10] Many case series of nosocomial infections attributed to coryneform bacteria are in the medical literature and include intravascular catheter–associated septicemia, native and prosthetic valve endocarditis, device-related infections,

TABLE 205.1 Community-Acquired Coryneform Infections

Conjunctivitis or keratitis	*Corynebacterium macginleyi* *Corynebacterium propinquum* *Corynebacterium pseudodiphtheriticum*
Pharyngitis	*Arcanobacterium haemolyticum* *Corynebacterium ulcerans* *C. pseudodiphtheriticum*
Peritonsillar and pharyngeal abscess	*A. haemolyticum*
Odontogenic infections	*A. haemolyticum* *Rothia dentocariosa*
Lymphadenitis	*Corynebacterium pseudotuberculosis*
Genitourinary tract infection	*Corynebacterium glucuronolyticum* *Corynebacterium riegelii*
Chronic prostatitis	*C. glucuronolyticum*
Skin and soft tissue infections	*A. haemolyticum* *Trueperella pyogenes* *Corynebacterium minutissimum* *C. pseudotuberculosis* *Corynebacterium confusum* *C. ulcerans*
Breast abscess	*Corynebacterium kroppenstedtii* *Corynebacterium tuberculostearicum* *C. minutissimum*
Native valve endocarditis	*A. haemolyticum* *R. dentocariosa* *C. pseudodiphtheriticum* *C. propinquum*
Native joint infection	*Corynebacterium striatum*

TABLE 205.2 Nosocomial Infections Caused by Coryneform Bacteria

Cerebrospinal fluid shunt infections	*Corynebacterium jeikeium*
Meningitis	*C. jeikeium* *Brevibacterium* spp.
Pneumonia	*Corynebacterium amycolatum (Corynebacterium xerosis)* *Corynebacterium striatum* *Corynebacterium urealyticum* *Corynebacterium pseudodiphtheriticum*
Intravenous catheter–related bloodstream infection	*C. jeikeium* *C. amycolatum* *C. striatum* *C. urealyticum* *Brevibacterium casei* *Corynebacterium macginleyi* *Corynebacterium minutissimum* *Corynebacterium afermentans* subsp. *afermentans* *Trueperella bernardiae* *Trueperella pyogenes* *Oerskovia* spp. *Microbacterium* spp.
Native valve endocarditis	*C. amycolatum* *C. jeikeium* *C. striatum* *C. urealyticum*
Prosthetic valve endocarditis	*C. jeikeium* *C. amycolatum* *C. striatum* *B. casei*
Skin and soft tissue infection	*C. amycolatum* *C. minutissimum* *C. urealyticum*
Postsurgical infections	*C. jeikeium* *C. urealyticum* *C. striatum* *C. minutissimum* *C. amycolatum*
Prosthetic joint infections	*C. jeikeium*
Urinary tract infections and encrusted cystitis	*C. urealyticum*
Continuous ambulatory peritoneal dialysis–related peritonitis	*C. jeikeium* *Brevibacterium* spp. *C. urealyticum* *Dermabacter* *Rothia dentocariosa*

peritonitis in peritoneal dialysis patients, and postoperative surgical site infections.[9,11,12] Common nosocomial pathogens include *Corynebacterium jeikeium, C. urealyticum, Corynebacterium amycolatum*, and *Corynebacterium striatum* (Table 205.2).[13] Nosocomial infections with the coryneform bacteria will continue to increase, reflecting the increased numbers of severely ill patients with extended stays in intensive care units and multiple antibiotic exposures.

Taxonomy

The taxonomy of the coryneform bacteria has evolved extensively over the past 30 years and continues to be refined. Hollis and Weaver,[14] at the Special Bacteriology Laboratory, Centers for Disease Control and Prevention (CDC) in Atlanta, completed the first extensive compilation of coryneform bacteria isolated from clinical specimens. Coryneform bacteria were grouped based on colony and biochemical characteristics. Since then, further work has been done to analyze these groups and define species. Table 205.3 lists the significant coryneform bacteria and the CDC group to which they previously belonged.

To date, more than 90 species of *Corynebacterium* have been identified; more than 50 species have been associated with disease in humans.[3,15,16] The use of molecular genetics has resulted in continued revision of the taxonomy of the coryneform bacteria and provides useful information on the epidemiology and pathogenicity of the genera. Molecular genetic studies, such as 16S rRNA and *rpoB* gene sequencing, are used in reference laboratories to confirm identification at the species level; 16S rRNA gene sequencing has become the standard by which new species are identified.[2,3,16–18] Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is another analytical test that is being used for identification of *Corynebacterium* spp.[19] With the MALDI-TOF technique, a mass spectrometer is used to analyze proteins that are extracted from the bacteria, and they are compared with a database.

Microbiology

Because the coryneforms are frequently cultured in polymicrobial infections and may be contaminants in cultures collected with poor sterile technique, clinician communication with the microbiology laboratory is essential in order to determine when species identification is appropriate. The decision to identify the coryneform bacteria to the species level is recommended when (1) the bacteria are cultured from normally sterile sites, such as blood (two or more positive blood cultures, except when recovered from the same set) or cerebrospinal fluid (CSF), (2) if the bacteria appear in adequately collected clinical material as the predominant organism on Gram staining and have a strong inflammatory reaction, or (3) are from urine specimens in which the bacterium (e.g., *C. urealyticum*) is the only organism recovered with a colony count greater than 10^4/mL *or* if it is the predominant bacterium cultured and the total bacterial count is greater than 10^5/mL.[3,16]

Media used for initial specimen processing are standard blood agar plates for most specimens, thioglycolate broth for wound cultures, and standard blood culturing systems using continuous monitoring for carbon dioxide (CO_2) production. Special media used for species identification include sheep blood agar supplemented with Tween 80, to assess lipid-enhanced growth.[3]

Identification to the species level in the microbiology laboratory is confirmed by biochemical testing. Initial testing includes the catalase test with 3% hydrogen peroxide. Additional tests include oxidation or fermentation; nitrate reduction; urea hydrolysis; esculin hydrolysis; and acid production from glucose, maltose, sucrose, mannitol, and xylose. A frequently used system of biochemical testing for medically relevant coryneform bacteria is the API Coryne system (API-bioMérieux, Marcy

TABLE 205.3 Medically Relevant Coryneform Bacteria

CLASSIFICATION	CDC CORYNEFORM GROUP
Nonlipophilic, fermentative corynebacteria	
Corynebacterium ulcerans	C. diphtheriae group
C. pseudotuberculosis	C. diphtheriae group
C. xerosis	F-2, I-2
C. striatum	I-1
C. minutissimum	
C. amycolatum	F-2, I-2
C. glucuronolyticum	
Others: *C. argentoratense, C. matruchotii, C. riegelii, C. confusum, C. simulans, C. sundsvallense, C. thomssenii, C. freneyi, C. aurimucosum, C. tuscaniae, C. coyleae, C. canis, C. falsenii, C. freiburgense, C. massiliense, C. pilbarense, C. stationis,* and *C. timonense*	
Nonlipophilic, nonfermentative corynebacteria	
C. afermentans subsp. *afermentans*	ANF-1
C. auris	
C. pseudodiphtheriticum	D-1
C. propinquum	ANF-3
Lipophilic corynebacteria	
C. jeikeium	JK
C. urealyticum	D-2
Others: *C. afermentans* subsp. *lipophilum, C. accolens, C. macginleyi, C. tuberculostearicum, C. kroppenstedtii, C. bovis,* CDC coryneform group F-1, *C. lipophiloflavum*	6 *(C. accolens)* G-1 *(C. macginleyi)* G-2 *(C. tuberculostearicum)*
Arcanobacteria	
Arcanobacterium haemolyticum	
Trueperella pyogenes (Arcanobacterium pyogenes)	
Trueperella bernardiae (Arcanobacterium bernardiae)	2
Other coryneform bacterial genera: *Turicella, Arthrobacter, Brevibacterium, Dermabacter, Rothia, Oerskovia, Microbacterium, Leifsonia aquatica*	A-1, A-2 (*Oerskovia* spp.) A-4, A-5 (*Microbacterium* spp.) B-1, B-3 *(Brevibacterium casei)* 3, 5 *(Dermabacter hominis)*
Rhodococci	

CDC, Centers for Disease Control and Prevention.

l'Étoile, France), which includes 20 biochemical tests and enables identification of many of the important corynebacteria and other coryneform bacteria, including *Arcanobacterium* spp. and *Brevibacterium* spp., and *Rhodococcus equi.*[20] An evaluation of the API Coryne database 2.0 found correct identification of 90.5% of the coryneforms tested.[21] Another identification system, RapID CB Plus (Thermo Fisher Scientific, Waltham, MA), correctly identifies 80.9% of strains to the species level and an additional 12.2% to the genus level. It has the advantage of requiring only 4 hours to perform, compared with 24 hours for the API Coryne system.[22] In a few cases, the Christie-Atkins-Munch-Petersen (CAMP) test helps to identify the organism to the species level.[2] MALDI-TOF has been used in comparison with biochemical identification for *Corynebacterium* species and coryneform bacteria. Comparison with sequencing of 16S ribosomal RNA genes demonstrated higher rates of identification with MALDI-TOF at the genus and species level for both *Corynebacteria* spp. and coryneform-like bacteria.[23]

The Clinical and Laboratory Standards Institute (CLSI) released standards for susceptibility testing of coryneform bacteria in 2016.[24] Isolates generally show susceptibility to vancomycin, daptomycin, and linezolid. Susceptibility to newer agents such as oritavancin, telavancin, and tedizolid has also been demonstrated.[25,26] Species of *Corynebacterium* are capable of expressing the *ermX* methylase gene, which is linked to the resistance phenotype macrolide, lincosamide and streptogramin B (MLS_B); this phenotype confers resistance to erythromycin and clindamycin and is associated with cross-resistance to other antimicrobial agents.[27] The *vanA* gene has been identified in *Oerskovia turbata* and *Arcanobacterium haemolyticum,* but no documented infections with vancomycin-resistant coryneforms have appeared in the literature.[28] When a clinically important isolate is obtained, susceptibility testing is recommended in order to ensure antimicrobial activity.

For consistency, the coryneform bacteria are reviewed here within groups identified by the presence or absence of lipid-enhanced culture (lipophilic or nonlipophilic) and fermentation activity.

Nonlipophilic, Fermentative Corynebacteria

Corynebacteria have been divided into lipophilic and nonlipophilic, fermentative and nonfermentative. Lipophilic species have enhanced growth in the presence of certain lipids, such as Tween 80. Fermentative strains produce acid from certain sugars. Advances made in the identification of species in the nonlipophilic fermentative group have resulted in a revision of thinking regarding the pathogenic role of several species, particularly *Corynebacterium xerosis* and *C. amycolatum.*[29] Interpretation of the literature that does not include detailed information on laboratory identification is difficult because of historical misidentification of species in the nonlipophilic fermentative group.

Corynebacterium ulcerans and *Corynebacterium pseudotuberculosis*

C. ulcerans and *C. pseudotuberculosis* are members of the *C. diphtheriae* group and are known primarily as animal pathogens, although disease in humans has been reported as zoonotic infections. Both *C. ulcerans* and *C. pseudotuberculosis* may elaborate diphtheria-like toxin.

Increasingly, *C. ulcerans* has been implicated in causing diseases such as exudative pharyngitis and cutaneous ulcers in humans indistinguishable from *C. diphtheriae.*[30,31] In the United Kingdom, *C. ulcerans* exceeded *C. diphtheriae* as the causative agent in diphtheria infection; in total, 59 cases of toxigenic *C. ulcerans* infection were reported from 1986 to 2008, and 12 cases from 2007 to 2013.[32,33] The European-based Diphtheria Surveillance Network reported an increase in diphtheria cases attributable to *C. ulcerans* during a 9-year surveillance period.[34] *C. ulcerans* has been implicated in human infection because of contact with domesticated animals; the use of multilocus sequence typing has allowed for rapid confirmation of zoonotic transmission.[35–37] This has made the identification of the causative organism important for epidemiology, and guidelines for laboratory diagnosis of diphtheria cases have been published; molecular testing with techniques such as real-time polymerase chain reaction (PCR) facilitates identification of toxigenic corynebacteria.[38,39] The spectrum of illness with *C. ulcerans* is similar to that with *C. diphtheriae.*[30,31] Fatalities have been reported, including sudden death from toxin-induced cardiac injury and a case of fatal necrotizing sinusitis.[40] Skin infection with *C. ulcerans* mimics that with *C. diphtheriae.*[41] Infection of the lower respiratory tract may occur, causing pneumonia and pulmonary nodules.[42,43] One case of possible human-to human transmission of *C. ulcerans* has been reported.[44] Treatment of pharyngitis caused by *C. ulcerans* is similar to treatment of diphtheria, including the use of antibiotics such as erythromycin and diphtheria antitoxin when appropriate.

C. pseudotuberculosis is a significant pathogen in animals, particularly sheep, in which it causes caseous lymphadenitis. Human disease is rare, manifesting as granulomatous lymphadenitis, found mainly in farm workers and veterinarians who have had exposure to infected animals.[45] It has been reported to cause a diphtheria-like illness and pneumonia, and has also been isolated from soft tissue abscesses in a young butcher.[32,46,47] Management of *C. pseudotuberculosis* infection includes excision of affected lymph nodes and treatment with β-lactam antibiotics, macrolides, or tetracyclines.

Corynebacterium xerosis

C. xerosis is a colonizer of the human nasopharynx, conjunctiva, and skin.[48] Historically, *C. xerosis* has been described in the literature as a pathogen that causes serious human disease, especially in immunocompromised hosts, including sepsis, endocarditis, pneumonia, peritonitis, ventriculoperitoneal shunt infection, and postoperative sternal wound infection. Subsequent investigations have questioned the reliability of identification of *C. xerosis* in the microbiology laboratory.[49,50] In one study, all isolates originally identified as *C. xerosis* were in actuality *C.*

amycolatum.[50] This calls into question preceding case reports attributing disease to *C. xerosis* because true *C. xerosis* isolates apparently are quite rare. *C. xerosis* infections in humans have included blepharitis, a brain abscess, and a case of sepsis in a pediatric patient with sickle cell disease.[51–53] True *C. xerosis* strains are susceptible to most antibiotics, which helps to distinguish them from *C. amycolatum,* which demonstrates multiple antibiotic resistances.

Corynebacterium striatum

C. striatum has been one of the more commonly isolated coryneform bacteria in the clinical microbiology laboratory.[2,3] As with other nonlipophilic fermentative corynebacteria, a high degree of misidentification of *C. striatum* has occurred in the past in microbiology laboratories, and investigators have found many isolates to be *C. amycolatum* on detailed retesting.[49,54]

C. striatum is ubiquitous and colonizes the skin and mucous membranes of normal hosts and hospitalized patients. Historically, *C. striatum* was not routinely identified to the species level and was often considered a contaminant. With the use of analytic tests such as MALDI-TOF, *C. striatum* is increasingly being recognized as an emerging pathogen.[55] Reports of true infection confirmed with isolation of *C. striatum* have increased in frequency and have been described in patients with indwelling devices, chronic pulmonary disease, and immunosuppression.[56–58] In addition, significant *C. striatum* infection has been reported in immunocompetent hosts. Case reports in the literature include native and prosthetic valve endocarditis, meningitis, pulmonary nodules, necrotizing fasciitis, septic arthritis, tubo-ovarian abscess, empyema, and osteomyelitis.[59–67] There is evidence for patient-to-patient transmission of *C. striatum* in hospital settings, which may account for the frequency with which it is isolated in hospitalized patients.[68,69] *C. striatum* has the ability to produce biofilm and has been implicated in a nosocomial outbreak of a multidrug-resistant strain.[70] Nosocomial outbreaks of *C. striatum* have been reported in patients with chronic obstructive pulmonary disease.[71]

Historically, *C. striatum* has been shown to be uniformly susceptible to vancomycin and other antimicrobials with broad gram-positive activity.[72] Resistance has been demonstrated to penicillin, ciprofloxacin, erythromycin, clindamycin, and tetracyclines, limiting potential oral antimicrobial options for therapy.[73,74] Increasingly, resistance to daptomycin has been reported; specifically in the setting of prior daptomycin therapy and during therapy in patients with infections of left ventricular assist devices.[75–77]

Corynebacterium minutissimum

Defined in 1983 by Collins, *C. minutissimum* is a colonizer of human skin, particularly moist intertriginous areas.[78] As with other members of this group, *C. amycolatum* has been misidentified as *C. minutissimum* in the past.[79] Although *C. minutissimum* historically has been considered the causative agent in erythrasma, that association has been questioned because cultures tend to show polymicrobial infection.[2] Erythrasma is a superficial skin infection that occurs in intertriginous areas between skin folds, axillae, groin, and fingers and toes.[10] It causes reddened scaling patches that may be accompanied by pruritus. Skin patches glow coral-red under a Wood lamp. Diagnosis is made by means of clinical appearance and symptoms and by culture of skin scrapings. Colonies also appear coral-red under ultraviolet light. Treatment includes topical and oral antibiotics. Recurrences are frequent.

Other rare infections attributed to *C. minutissimum* include septicemia and endocarditis in immunocompromised patients and patients with indwelling central venous catheters, peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD), pyelonephritis, costochondral abscess, breast abscess, postoperative abdominal infection, and vascular graft infection.[80–82] One case of bacteremia and meningitis has been reported, in addition a case of an infected pseudomeningocele.[83,84]

Corynebacterium amycolatum

Defined as a new species in 1988 by Collins, *C. amycolatum* was first isolated from the skin of healthy humans.[85] Noted for its lack of mycolic acids, the species corresponds to the CDC coryneform groups F-2 and I-2. It is the nonlipophilic coryneform bacterial species most frequently isolated from clinical specimens.[7,8] *C. amycolatum* forms small dry nonhemolytic colonies of 1 to 2 mm in diameter when cultured at 37°C.[3] The organisms are pleomorphic and vary from single organisms to an array of Chinese letters. Because of variability in biochemical reactions, *C. amycolatum* had been misidentified previously as *C. minutissimum, C. xerosis,* and *C. striatum.* Currently, the API Coryne system can correctly identify *C. amycolatum,* but confirmatory tests should be performed.[3]

Although case reports of infections attributed to *C. amycolatum* are rare, many previously reported infections by other members of the nonlipophilic fermentative group were most likely caused by *C. amycolatum.* Reports with reliable information on organism identification include nosocomial endocarditis after intravenous catheter–related infection, septic arthritis, a case of native valve endocarditis with aorta–left atrial fistula, and sepsis in pediatric oncology patients.[86–88] *C. amycolatum* has been implicated in ear infections and orbital implant infections.[89,90] Susceptibility testing has shown resistance to penicillins, cephalosporins, macrolides, and fluoroquinolones, and susceptibility to vancomycin, daptomycin, and linezolid.[91] There is variable resistance to aminoglycosides and tetracyclines.[92,93] Reports of successful treatment of endovascular infection include the use of vancomycin and daptomycin in combination with rifampin.[94]

Corynebacterium glucuronolyticum

C. glucuronolyticum was defined in 1995; since 2000, the species has included isolates previously identified as *Corynebacterium seminale* that had been defined by Riegel and coworkers.[95,96] Although it has been primarily isolated from the genitourinary tract of animals, in humans it may be included in the normal flora of the genitourinary tract. It is commonly isolated from males with genitourinary tract infections and is associated with chronic prostatitis; a case of encrusted cystitis due to *C. glucuronolyticum* has been reported.[97–99] *C. glucuronolyticum* strains are susceptible to β-lactam antibiotics, gentamicin, and vancomycin but demonstrate variable resistance to fluoroquinolones, macrolides, and tetracyclines.[92]

Other Nonlipophilic, Fermentative Corynebacteria

Corynebacterium argentoratense has been isolated from the throats of healthy volunteers and from mucosal biofilms on adenoid tissue from children with chronic or recurrent otitis media. The clinical significance of this finding is unclear.[100] *Corynebacterium matruchotii* is identified by its characteristic "whip handle" appearance on Gram staining.[2,3] It was previously identified as *Bacterionema matruchotii* until 1983, when it was reclassified as a *Corynebacterium* species by Collins. Mainly an inhabitant of the oral cavity of humans and animals, *C. matruchotii* has been rarely associated with human disease.

In 1998, Funke and colleagues[101] identified a new species of *Corynebacterium* isolated from female patients with symptomatic urinary tract infections. Given the name *Corynebacterium riegelii,* it is nonlipophilic, weakly fermentative, and facultatively anaerobic. Similar to the lipophilic *C. urealyticum,* it demonstrates strong urease activity. It is susceptible to penicillins, cephalosporins, gentamicin, fluoroquinolones, and tetracyclines.

Corynebacterium confusum was defined in 1998 by Funke and colleagues[29]; it is nonlipophilic and very slowly fermentative.[102] *C. confusum* has been isolated from a blood culture, foot infections, and a breast abscess.[102] Additional nonlipophilic fermentative *Corynebacterium* spp. identified from human clinical specimens include *C. simulans, C. sundsvallense, C. thomssenii, C. freneyi, C. aurimucosum, C. tuscaniae, C. coyleae, C. canis, C. falsenii, C. freiburgense, C. massiliense, C. pilbarense, C. stationis,* and *C. timonense.*[103–114]

Nonlipophilic, Nonfermentative Corynebacteria

The nonlipophilic, nonfermentative corynebacteria do not produce acid from any sugars and were designated as absolute nonfermenters (ANFs) by Hollis and Weaver.[14] They are colonizers of the human respiratory tract and ear canal and are infrequent pathogens.

Corynebacterium afermentans subsp. *afermentans*

C. afermentans subsp. *afermentans* was included in the CDC coryneform group ANF-1 until 1993, when Riegel and coworkers[115] defined the species as *C. afermentans* with two subspecies: *C. afermentans* subsp. *afermentans* and *C. afermentans* subsp. *lipophilum. C. afermentans* subsp. *afermentans* is a rare human pathogen but has been reported to cause septicemia in immunocompromised patients.[116]

Corynebacterium auris

As in the case of *Turicella otitidis, C. auris* was initially isolated from middle ear fluid of pediatric patients with otitis media and was presumed to be among the pathogens that cause otitis media. Subsequent studies have cultured *C. auris* from the external ear canal and cerumen of healthy subjects, both children and adults, and its role as a pathogen has been discounted.[5,117] *C. auris* is resistant to penicillins, clindamycin, and erythromycin and susceptible to fluoroquinolones, gentamicin, tetracyclines, and vancomycin.[92]

Corynebacterium pseudodiphtheriticum

C. pseudodiphtheriticum is included in the normal bacterial flora of the human upper respiratory tract. Lehmann and Neumann[1] described the organism in 1896, giving it the name *Bacillus pseudodiphtheriticum.* Since 1925, it has been known as *C. pseudodiphtheriticum.* Historically, *C. pseudodiphtheriticum* was associated with endocarditis of native and prosthetic valves.[118] The first cases of infections at other sites attributable to *C. pseudodiphtheriticum* became known in 1982, and since then, *C. pseudodiphtheriticum* has been associated primarily with respiratory infections, particularly in immunocompromised hosts and in patients with chronic lung disease.[119,120] It has been isolated from patients with pneumonia and advanced acquired immunodeficiency syndrome (AIDS), and from children with cystic fibrosis and respiratory infections.[121,122] Other sites of infections have been the eye, intervertebral disks, joints, lymph nodes, urine, peritoneal fluid, intravenous catheters, and surgical wounds.[123] Although *C. pseudodiphtheriticum* does not elaborate toxins, it has been isolated from patients with exudative pharyngitis with pseudomembrane formation, not unlike *C. diphtheriae.*[124] Corneal scrapings from patients with bacterial keratitis due to *C. pseudodiphtheriticum* were evaluated for host immune response to infection; elevated expression of Toll-like receptors and proinflammatory cytokines interleukin (IL)-6 and Il-1β were noted.[125] Isolates of *C. pseudodiphtheriticum* have demonstrated resistance to macrolides and lincosamides but have maintained susceptibility to penicillins, cephalosporins, doxycycline, and glycopeptides. One large case series of 113 *C. pseudodiphtheriticum* strains from a single institution showed moderate levels of resistance to β-lactams, imipenem, tetracycline, erythromycin, ciprofloxacin, aminoglycosides, and clindamycin; all strains were susceptible to vancomycin.[123]

Corynebacterium propinquum

Before 1994, *C. propinquum* was known as CDC coryneform group ANF-3; it is primarily isolated from the human respiratory tract.[120,126] *C. propinquum* has been implicated in native and prosthetic valve endocarditis; species identification was confirmed with 16s rRNA gene sequencing and MALDI-TOF.[127,128] *C. propinquum* has also been isolated from a pulmonary pleural effusion, an infected orthopedic device, and a plaque associated with keratitis in a diabetic patient who used a therapeutic contact lens; a case of nongonococcal urethritis due to *C. propinquum* has been reported.[129,130]

Lipophilic Corynebacteria

Lipophilic corynebacteria are fastidious, slow-growing bacteria that form tiny nonhemolytic colonies on standard media but demonstrate enhanced growth with the addition of lipids to the culture medium.[2] The group includes the significant human pathogens *C. jeikeium* and *C. urealyticum.*

Corynebacterium jeikeium

C. jeikeium was initially described in 1976 as a highly resistant coryneform bacteria that caused severe sepsis in patients with hematologic malignancies and profound neutropenia and in one patient with a ventricular CSF shunt.[131] In 1979, it was designated as CDC group JK, and in 1988, the designation was revised to *C. jeikeium.*[132] Whole-genome sequencing has revealed that *C. jeikeium* is actually a group of four genomospecies.[133] *C. jeikeium* colonizes the skin of hospitalized patients, especially those treated with multiple antibiotics, and can also be isolated from the hospital environment.[134] There is some evidence that patient-to-patient transmission occurs in the hospital. It is the most frequently isolated *Corynebacterium* in the acute-care setting and is the most important pathogen of the lipophilic corynebacteria.[7]

Microbiology

C. jeikeium is a pleomorphic gram-positive rod that varies in form from coccobacillary to bacillary; some appear club shaped. It is nonhemolytic on standard media and forms small gray-white colonies on routine culture.[2] It is lipophilic and forms large colonies on sheep blood agar supplemented with Tween 80. *C. jeikeium* does not produce urease or reduce nitrate, and it ferments glucose.

Pathogenicity

C. jeikeium is a cause of severe infections in the hospitalized patient.[135] Predisposing factors for infection include immunocompromised states such as malignancy, neutropenia, and AIDS.[135,136] Other risk factors include the presence of indwelling medical devices such as central venous catheters, peritoneal dialysis catheters, prosthetic valves, and CSF shunts. Prolonged hospital stay, treatment with broad-spectrum antibiotics, and impaired skin integrity are well-described risk factors for development of infection with *C. jeikeium.*

Infectious processes include septicemia from infected intravascular devices, native and prosthetic valve endocarditis, CSF shunt infections, meningitis and transverse myelitis, and prosthetic joint infections.[137,138] It has been reported to cause postsurgical infections, peritonitis in patients who undergo CAPD, liver abscess, malignant otitis externa, and osteomyelitis; glomerulonephritis attributable to left ventricular assist device infection has been reported.[139,140] Skin findings with *C. jeikeium* infection are common: neutropenic patients with *C. jeikeium* septicemia commonly have reported skin findings including rash and subcutaneous nodules.[141,142]

Treatment

C. jeikeium is resistant to many antibiotics, including penicillins, cephalosporins, and aminoglycosides; there is inducible resistance to macrolides.[143,144] It remains susceptible to vancomycin, which is the recommended treatment. Although catheter removal has been routinely recommended in the setting of intravascular catheter–related infection, experience has shown a high success rate in catheter salvage with appropriate antimicrobial therapy.[145] Successful treatment with daptomycin and tigecycline have been reported; one case of a daptomycin-resistant strain of *C. jeikeium* in a previously treated neutropenic patient has been reported.[146–148]

Corynebacterium urealyticum

First described in 1974, this bacterium was designated as CDC group D2 until 1992, when the name *C. urealyticum* was proposed.[149] *C. urealyticum* colonizes the skin of 25% to 37% of hospitalized patients. Because of its ability to adhere to uroepithelial cells, it is most commonly associated with urinary tract infections, especially in cases of abnormal anatomy, and has been implicated as the cause of encrusted cystitis and encrusted pyelitis.[150]

Microbiology

Colonies of *C. urealyticum* are slow growing and lipophilic and appear nonhemolytic and pinpoint when cultured on sheep blood agar under CO_2 enrichment for 48 hours.[2] It is a strict aerobe, with no growth under anaerobic conditions. On Gram staining, organisms are palisading, non–spore-forming coccobacilli. They are catalase positive and oxidase negative, with a rapid production of urease. Laboratories should be made aware of the need for further investigation of diphtheroid bacilli from urinary tract specimens in the proper clinical setting because *C. urealyticum* may not grow in standard urine culture.[2]

Pathogenicity

C. urealyticum is primarily a cause of chronic and recurrent urinary tract infections, occurring mainly in elderly people and those with debilitation or immunosuppression. Additional risk factors include prolonged hospitalization, the use of percutaneous and bladder drainage catheters, and urinary tract procedures.[151,152] It has been reported to cause infections in renal transplant recipients.[153] Clues to diagnosis of *C. urealyticum* infection include sterile pyuria, alkaline urine, and the presence of white blood cells and struvite crystals.[154] *C. urealyticum* causes encrusted cystitis, which appears as chronic inflammation of the bladder mucosa with crystal deposits on the bladder mucosa, surrounded by erythema. Encrusted pyelitis may occur if there are abnormalities of the upper urinary tract. In rare cases, *C. urealyticum* has been reported as a causative agent in peritonitis, endocarditis, pneumonia, septicemia, osteomyelitis, soft tissue infections, and superinfection of wounds.[151,152,155]

Treatment

In general, *C. urealyticum* is resistant to β-lactams, aminoglycosides, and trimethoprim-sulfamethoxazole. There is variable susceptibility to fluoroquinolones, macrolides, and tetracycline.[7,151,155] The treatment of choice is vancomycin, to which it remains susceptible. For urinary tract infections, in addition to vancomycin, endoscopic removal of bladder mucosa encrustations or acidification of urine by instillation of acid into the bladder in cases of encrusted cystitis may be required, and urologic consultation is recommended.[156] The use of percutaneous nephrostomy tube placement and irrigation of the upper urinary tract with Thomas acid solution, in cases of upper tract disease, has been described.[157]

Other Lipophilic Corynebacteria

C. afermentans subsp. *lipophilum* is a rarely reported human pathogen.[115] It has been reported to cause intravascular catheter–related septicemia, prosthetic valve endocarditis, lung abscess, empyema, and brain abscess. *Corynebacterium accolens* was previously known as CDC coryneform group 6. There were discrepancies in the definition until 1991, when it was defined further by Neubauer and associates[158] and given the name *Corynebacterium accolens.* Known to colonize the human upper respiratory tract, *C. accolens* is a rarely reported human pathogen but has been reported to cause septicemia, endocarditis, breast abscess, and pelvic osteomyelitis.[159–161] As compared with controls, infants with cystic fibrosis had higher levels of *C. accolens* in the nasopharyngeal microbiota than of other *Corynebacterium* spp.[162] *Corynebacterium macginleyi* (formally CDC coryneform group G-1) was initially isolated solely from the human eye as a cause of conjunctivitis.[163] In an 8-year survey at a single institution, *C. macginleyi* was identified as the causative agent of microbial keratitis in 20% of patients with this condition.[164] Case reports of *C. macginleyi* infection include intravascular catheter–associated bloodstream infection, urinary tract infection associated with a bladder drainage catheter, ventilator-associated pneumonia in a lung cancer patient, and septicemia in immunocompromised patients.[165,166] Other lipophilic corynebacteria, including *Corynebacterium tuberculostearicum* (formally CDC coryneform group G-2) and *Corynebacterium kroppenstedtii,* have been cultured from inflammatory breast tissue in cases of granulomatous mastitis; in particular, *C. kroppenstedtii* has been reported in multiple case series in association with inflammatory breast disease.[167–170] *C. kroppenstedtii* was identified as a cause of cystic neutrophilic granulomatous mastitis, a condition that is characterized by lipogranulomas with cystic spaces surrounded by neutrophils.[171] The first case positing a causal role for *C. kroppenstedtii* in granulomatous mastitis occurred in a patient with abnormal neutrophil responses to a NOD2 agonist.[172]

A case series from a single center reviewed 16 patients with granulomatous mastitis due to *Corynebacterium* spp.; *C. kroppenstedtii* was the most frequently identified organism, followed by *C. tuberculostearicum.* Susceptibility testing showed that *C. kroppenstedtii* was resistant to β-lactam antibiotics, whereas *C. tuberculostearicum* was multidrug resistant.[168] A strain of multidrug-resistant *C. kroppenstedtii*–carrying multiple resistance genes has been described in a patient with granulomatous mastitis.[173] *C. kroppenstedtii* has been identified as the causative agent in a case of prosthetic valve endocarditis.[174] *C. tuberculostearicum* has also been associated with postsurgical deep wound infections and osteomyelitis.[175] CDC *Corynebacterium* group G has caused native and recurrent prosthetic valve endocarditis.[176] *Corynebacterium bovis* is a cause of bovine mastitis, but in humans has been described as a cause of endocarditis, chronic otitis media, central nervous system (CNS) infection, line-related septicemia, and joint infection.[177–179] CDC coryneform group F-1 may be a cause of urinary tract infection; it is similar to *C. urealyticum* in its very rapid urease reaction and differs from the latter in its very high susceptibility on antimicrobial testing.[180] *Corynebacterium lipophiloflavum* has been isolated from a patient with bacterial vaginosis. *Corynebacterium resistens* is a multidrug-resistant coryneform bacteria isolated from blood, bronchial aspirate, and abscess specimens.[181] *Corynebacterium ureicelerivorans* has been implicated in bacteremia and peritonitis.[182,183] New species of lipophilic coryneform bacteria found in human specimens continue to be defined; these include *Corynebacterium aquatimens, Corynebacterium sputi,* and *Corynebacterium pyruviciproducens.*

Arcanobacteria

Collins defined the genus *Arcanobacterium* in 1982, from *arcane,* meaning "mysterious or secret," and *bacterium.*[184] For many years, *A. haemolyticum* was the only species in this genus. However, in 1997, further investigation of several *Actinomyces* spp. resulted in the reclassification of *Actinomyces pyogenes* and *Actinomyces bernardiae* as *Arcanobacterium* spp. and defined two additional new species of arcanobacteria.[185] In 2011, *Arcanobacterium pyogenes* and *Arcanobacterium bernardiae* were reclassified to a new genus, *Trueperella,* and are currently identified as *Trueperella pyogenes* and *Trueperella bernardiae.*[186]

Arcanobacterium haemolyticum

A. haemolyticum was first isolated by MacLean and coworkers[187] in 1946 from American soldiers and Pacific Islanders with pharyngeal and skin infections in the South Pacific. The initial classification as *Corynebacterium haemolyticum* endured until 1982, when the genus *Arcanobacterium* was defined by Collins. *Corynbacterium haemolyticum* is no longer used.

Microbiology

A. haemolyticum is a catalase-negative, gram-positive to gram-variable rod that does not form spores and is nonmotile.[2] It is β-hemolytic, but expression can vary with culture media and conditions; hemolysis is best observed on human blood agar.[188] Growth is enhanced in the presence of CO_2. It is known for forming dark pits under the colonies. Poor growth on tellurite helps to differentiate it from *C. diphtheriae.*

Colony morphology has been described as either rough or smooth type.[189] Rough-type colonies are most frequently associated with respiratory isolates; smooth biotypes are most frequently associated with wound isolates. *A. haemolyticum* does not ferment xylose, which differentiates it from *T. pyogenes.* A positive α-mannosidase test can be used to identify *A. haemolyticum* and differentiate it from *T. pyogenes* and other coryneform-like bacteria, including *R. equi* and *Erysipelothrix rhusiopathiae.* Because of the presence of phospholipase D activity similar to *C. ulcerans* and *C. pseudotuberculosis,* the reverse CAMP test result will be positive, with inhibition of the hemolytic zone of a β-lysin–producing strain of *Staphylococcus aureus.* Other virulence factors include neuraminidase and hemolysin production.[190]

Infections in Humans

A. haemolyticum is a well-recognized cause of pharyngitis in humans, with a spectrum of illness from mild to diphtheria-like.[191–193] It accounts for about 0.5% of pharyngeal infections overall and 2.0% in individuals in the 15- to 25-year-old age range. In studies, *A. haemolyticum* has not been isolated from healthy control populations but has been isolated from 2.5% of a symptomatic young adult population.[193–195] It is indistinguishable from streptococcal pharyngitis in clinical appearance, and about 50% of cases of pharyngitis are exudative. Cervical adenopathy is usually present. *A. haemolyticum* pharyngitis is accompanied by an exanthem in about 50% of cases. The rash generally appears after the onset of the pharyngitis and has a variable appearance, often described as an erythematous morbilliform or scarlatiniform rash, appearing on

FIG. 205.1 Skin rash in a patient with *Arcanobacterium haemolyticum* pharyngitis.

the trunk, neck, and extremities (Fig. 205.1). It may also manifest as an erythematous urticarial rash with an appearance similar to that of erythema multiforme. Complications of *A. haemolyticum* pharyngitis include peritonsillar and pharyngeal abscesses, with *A. haemolyticum* being the sole pathogen in 50% of cases in adolescents and young adults, and the remaining 50% coinfected with β-hemolytic streptococci.[194]

A. haemolyticum has been isolated from soft tissue infections, including chronic ulcers, wound infections, cellulitis, and paronychia.[196] It is frequently a component of polymicrobial infection in this setting but has also been isolated as the sole pathogen.[197] Underlying conditions in polymicrobial chronic ulcers include diabetes and peripheral vascular disease. Posttraumatic wound infections have been reported, as has coinfection or superinfection with leprosy ulcers.[198]

Lemierre disease with *Fusobacterium necrophorum* and *A. haemolyticum* has been reported, accompanied by a skin rash typical for *A. haemolyticum* infection; Lemierre syndrome and septicemia caused by *A. haemolyticum* have also been reported.[199,200] Sepsis syndrome from *A. haemolyticum* has been described, occurring in all age groups and without predisposing factors.[201] Other infections reported include sinusitis, orbital cellulitis, orbital necrotizing fasciitis and osteomyelitis, brain abscess, endocarditis, cavitary pneumonia, and osteomyelitis.[202,203]

Treatment

Susceptibility information for *A. haemolyticum* has been reviewed extensively.[204] Although in vitro studies show most strains to be penicillin susceptible, treatment failures may occur because of tolerance and poor penetration into the intracellular space. Other β-lactams have also shown in vitro activity. Susceptibility data showed low minimal inhibitory concentrations to erythromycin and azithromycin.[204] Clindamycin and doxycycline are also efficacious, as are ciprofloxacin and vancomycin. Resistance to trimethoprim-sulfamethoxazole and tetracycline is well documented.[205] Surgical management of wound infections and drainage of soft tissue abscesses are recommended.

Trueperella (Arcanobacterium) pyogenes

Initially described by Glage in 1903, this organism was initially named *Bacillus pyogenes.* It was known as *Corynebacterium pyogenes* until 1982, when it was reassigned to the genus *Actinomyces.* In 1997, *Actinomyces pyogenes* was transferred to the genus *Arcanobacterium* and was renamed *Arcanobacterium pyogenes.*[185] In 2011, *Arcanobacterium pyogenes* was further reclassified as *Trueperella pyogenes.*[186] *T. pyogenes* is primarily an animal pathogen that causes pyogenic infections in cattle, including pneumonia, endometritis, endocarditis, wound infections, and mastitis. Abscess formation is aided by neuraminidases, which facilitate adhesion to host epithelial cells.[206] Transmission of *T. pyogenes* by flies has been proposed. *T. pyogenes* has not been isolated as normal human flora. Most human cases are acquired in rural settings and include outbreaks of leg ulcers in Thai children, septicemia in a patient with colon carcinoma, polymicrobial-infected diabetic foot ulcers, spondylodiskitis and psoas abscess, subcutaneous abscesses, and intraabdominal infections.[207,208] Cases of fatal endocarditis in patients with no animal contact have been reported.[209,210] *T. pyogenes* is cultured on sheep blood agar under CO_2 enrichment. Colonies are weakly hemolytic at 24 hours and become more strongly hemolytic at 48 hours.[2] Differentiation from *A. haemolyticum* is made with observation of the CAMP reaction, by fermentation of xylose, and by the α-mannosidase test. *T. pyogenes* is susceptible to most antibiotics, including penicillins, cephalosporins, macrolides, tetracyclines, and aminoglycosides.

Trueperella (Arcanobacterium) bernardiae

Originally described as CDC coryneform group 2 in 1987, this organism was assigned the species name *Actinomyces bernardiae* in 1995. In 1997, *Actinomyces bernardiae* was transferred to the genus *Arcanobacterium* as *Arcanobacterium bernardiae*; in 2011, *Arcanobacterium bernardiae* was further reclassified as *Trueperella bernardiae.*[185,186] On Gram staining, it appears as short gram-positive rods without branching. It is identified through its ability to ferment maltose more rapidly than glucose, which separates it from other coryneform bacteria. It is distinguished from *T. pyogenes* by the inability to ferment sucrose, mannitol, and xylose.[2] *T. bernardiae* is a rare human pathogen, with recovery of the organism from the bloodstream, abscesses, the urinary tract, joints, the eye, and wounds; it has also been implicated as a cause of necrotizing fasciitis and prosthetic joint infection.[211–214]

Miscellaneous Coryneform Bacteria

Turicella otitidis

Initially isolated from patients with otitis media, *T. otitidis* is believed to be a colonizer of the human auditory canal and not a true pathogen in this setting because it has been isolated in the same frequency from an asymptomatic control population.[5,117,215] It has been reported as a cause of mastoiditis and posterior auricular abscess in immunocompetent children and septicemia in a neutropenic child. *T. otitidis* is resistant to clindamycin and erythromycin but susceptible to penicillins, cephalosporins, tetracyclines, fluoroquinolones, linezolid, and vancomycin.[216]

Arthrobacter Species

An environmental coryneform found in animal sheds, schools, and daycare centers, *Arthrobacter* has rarely been isolated from human clinical specimens. Commonly identified species include *Arthrobacter cumminsi* and *Arthrobacter oxydans.*[217,218] There are reports of septicemia in immunocompromised patients and isolation of *Arthrobacter* from human urine specimens. Disseminated intravascular coagulation due to *Arthrobacter* septicemia in a pregnant patient was implicated as contributing to intrauterine fetal demise.[219]

Brevibacterium Species

Brevibacterium spp. are short coryneforms isolated from milk and dairy products and are known colonizers of human skin.[8] They have been identified in environmental dust in schools, daycare centers, and animal sheds. Brevibacteria have a biphasic morphologic appearance on culture, with young colonies demonstrating typical coryneform features. As colonies age, the organisms mature into cocci or a coccobacillary appearance.[2] Brevibacteria have been implicated in causing human foot odor when confining footwear results in a moist environment. Only a few species of *Brevibacterium* have been noted to cause infection.

B. casei is the species of this genus that is most frequently isolated from human clinical specimens.[220] On culture, it forms white-gray colonies with a distinctive cheese odor. On Gram staining, it is a short, club-shaped rod that is catalase positive and non–spore forming.[2,221] Human infections with brevibacteria have most frequently been

intravascular catheter–related bloodstream infections, particularly in immunocompromised patients and patients with AIDS. In one case series, six of 11 patients with pulmonary hypertension receiving continuous iloprost via a central venous catheter developed catheter-related infection due to *Brevibacterium* spp.[222] There have been additional reports of meningitis, brain abscess, cholangitis, salpingitis, and peritonitis in patients undergoing CAPD.[223,224] Susceptibility testing shows some resistance to β-lactam antibiotics, fluoroquinolones, clindamycin, and macrolides.[216,225] Vancomycin is the treatment of choice for serious infections.[225] Other *Brevibacterium* spp. that have been reported to cause invasive disease include *Brevibacterium sanguinis, Brevibacterium epidermidis,* and *Brevibacterium otitidis.*[226–228]

Dermabacter hominis

Dermabacter spp. were previously identified as CDC group 3 and group 5 coryneform bacteria and are skin colonizers of humans.[229] They have been a cause of bacteremia in patients with prolonged hospitalizations and peritonitis in immunocompromised persons who undergo CAPD. *Dermabacter* has been isolated from a cerebral abscess in a renal transplant recipient and from a patient with chronic osteomyelitis with *Actinomyces neuii* as copathogen.[230] *D. hominis* exhibits variable resistance to many antibiotics, including penicillins, fluoroquinolones, macrolides, chloramphenicol, and tetracyclines, and susceptibility to vancomycin and linezolid; high rates of resistance to daptomycin have been reported.[216,231]

Rothia dentocariosa and *Rothia mucilaginosa*

Rothia are found as colonizers of the human oral cavity and have been isolated from dental plaque and in cases of periodontal disease.[232] *R. dentocariosa* has the potential for misidentification as a *Dermabacter* or *Actinomyces* species in the microbiology laboratory.[2] Case reports with reliable information on identification of the organisms have found it to be a pathogen in several cases of native and prosthetic valve endocarditis, including presentations with abscesses, mycotic aneurysms, and vertebral osteomyelitis.[233,234] It has also been a cause of bacteremia without endocarditis.[234,235]

R. mucilaginosa, formerly *Stomatococcus mucilaginosus,* is a normal resident of the human mouth and nasopharynx. On culture, it usually appears as gram-positive cocci in clusters—hence, the previous classification as a *Stomatococcus. R. mucilaginosa* is a rare cause of true bacteremia and sepsis; two case series from large academic institutions each identified more than 20 patients with true *Rothia* bacteremia. *R. mucilaginosa* was identified as the predominant species recovered; the majority of patients had neutropenia and hematologic malignancy.[235,236] *R. mucilaginosa* has been found in cases of pneumonia in patients with leukemia and lung cancer and peritonitis in patients undergoing CAPD.[237,238] A case of granulomatous dermatitis attributable to *R. mucilaginosa* bacteremia has been reported.[239]

Oerskovia and *Cellulosimicrobium* Species

Included in CDC group A-1 and A-2, *Oerskovia* spp. are rare human pathogens but have been reported to cause infection in immunocompromised hosts, patients with implanted devices, and those with indwelling central venous catheters.[2] The spectrum of infections has ranged from bacteremia, endocarditis, meningitis associated with CSF shunt infection, soft tissue infection, prosthetic joint infection, and peritonitis in a patient undergoing CAPD.[240–242] Two species, *O. turbata* and *Oerskovia xanthineolytica,* have been reclassified as *Cellulosimicrobium funkei* and *Cellulosimicrobium cellulans,* respectively, based on 16S rRNA sequencing.[243] Infections due to *C. cellulans* include peritonitis in a patient who was undergoing CAPD, and pyogenic flexor tenosynovitis in a patient with traumatic injury due to introduction of a foreign body (wooden splinters) in one finger.[244,245]

Microbacterium Species

CDC coryneform group A-4 and A-5 bacteria were defined as *Microbacterium* spp., and in 1998 the genus *Aureobacterium* was reclassified and renamed within the genus *Microbacterium.*[246,247] *Microbacterium* spp. have been found as a cause of bacteremia, peritonitis in patients who undergo CAPD, and endophthalmitis.[248–250] Most commonly, it has been a nosocomial pathogen in debilitated and immunocompromised patients. In a study of 50 human isolates, the most common species recovered were *Microbacterium oxydans, Microbacterium paraoxydans,* and *Microbacterium foliorum.*[251]

Leifsonia aquatica

Corynebacterium aquaticum was reclassified in 2000 as *Leifsonia aquatica.*[252] Because of inconsistencies of identification and confusion with *Aureobacterium* in previous reports, it has been difficult to determine the pathogenicity of this species. Case reports for *L. aquatica* are rare; *L. aquatica* had been reported to cause septicemia in immunocompromised hosts, peritonitis in patients on CAPD, and bacteremia in a hemodialysis patient.[253] One case of septicemia due to *L. aquatica* after retinal detachment surgery has been reported.[254]

Other medically relevant coryneform bacteria

Other medically relevant coryneform bacteria include the genera Auritidibacter, Exiguobacterium, Cellulomonas, Helcobacillus, Curtobacterium, Janibacter, Pseudoclavibacter, Brachybacterium, and Knoellia.[3] Isolation of these rare organisms has typically been in the context of clinical material submitted to a microbiologic research laboratory for further characterization.

RHODOCOCCI

Taxonomy

Rhodococcus ("red coccus") belongs to the family Nocardiaceae, order Actinomycetes, which includes *Nocardia, Corynebacterium, Mycobacterium,* and *Gordonia* spp. This genus is made up of genetically and physiologically diverse bacteria that have environmental, clinical, and industrial significance. *R. equi* is the most commonly isolated species causing human infection, especially among immunocompromised hosts with defective cell-mediated immunity. Recent debate over taxonomy and nomenclature has led to a proposed reclassification of *R. equi* as *Rhodococcus hoagii* or *Prescottella equi.*[255] Other members of this genus that are human pathogens include *Rhodococcus rhodochrous, Rhodococcus fascians (Rhodococcus luteus),* and *Rhodococcus erythropolis.*

Rhodococcus equi

Epidemiology

R. equi (formerly *Corynebacterium equi*) was first identified as a pathogen in 1923, when it was isolated from the lungs of foals with pyogranulomatous pneumonia. It has subsequently been identified in a variety of animals, including cattle, swine, sheep, goats, deer, bears, wild birds, seals, dogs, and cats.[256] The first case of human infection was reported in 1967, when *R. equi* was cultured from lung specimens of a young man who worked in a stockyard, was being treated with corticosteroids and 6-mercaptopurine for autoimmune hepatitis, and presented with fever and cavitary pneumonia. During the next decade, sporadic cases of infection in humans were reported. Beginning in the early 1980s, the incidence of *R. equi* infection increased markedly. This increase has been attributed to the human immunodeficiency virus (HIV) infection epidemic, advances in chemotherapy for malignancies, and organ transplantation.[257,258] In addition, improvements in microbiology laboratory identification techniques and increasing recognition of *R. equi* as a pathogen may also explain part of the increase in incidence.[259] The frequency of *R. equi* infections in HIV-infected patients seems to have decreased in recent years, largely related to highly active antiretroviral therapy and possibly to prophylaxis with azithromycin. More than 200 cases of infection caused by *R. equi* have been published. *R. equi* has been isolated from water and soil worldwide and from the manure of herbivores.[256] Infection in both animals and humans is thought to be acquired through inhalation or ingestion of the organism. Inoculation into a wound can also lead to infection. Exposure to farm soil, animals, or manure has been reported in many human cases, although it is less common in HIV-positive patients.[260,261] *R. equi* has been rarely isolated from healthy persons without an identified immunosuppressive condition.[262,263] An environmental or occupational exposure (farmer, horse breeder) was identified in 50% of cases.[263] Most infected individuals have had defective cell-mediated immunity, including HIV infection, with or without a history of animal exposure. Health care–associated

cases of *R. equi* have been reported.[264] Human-to-human transmission has been suspected in cases of *R. equi* pneumonia acquired by HIV-infected patients who were roommates of patients infected with *R. equi.*[265] Occupational acquisition of *R. equi* by a healthy laboratory worker who developed pneumonia has been reported. *Rhodococcus* spp. with properties very similar to those of *R. equi* have been isolated as nasal flora in adults.[266]

Microbiology

R. equi is a gram-positive obligate aerobe that is asporogenous and nonmotile. It may appear coccoid or bacillary, depending on growth conditions. Its bacillary appearance varies from long, curved, clubbed forms to short filaments with branching. *R. equi* can grow at a variety of temperatures but grows optimally at 30°C. Colonies on solid media appear large, irregular, smooth, and mucoid. They are pale salmon-pink in color; however, this characteristic color may not appear until days 4 to 7 of incubation. Although it grows well on ordinary media, if cultured in this manner the organism may be overlooked or discarded as a nonpathogenic coryneform or misidentified as *Nocardia* or *Micrococcus.* Isolation of *R. equi* from contaminated specimens is facilitated by the use of selective media, such as colistin-nalidixic agar, phenylethyl alcohol agar, or ceftazidime-novobiocin agar. *R. equi* is catalase, lipase, urease, and phosphatase positive. It is oxidase, elastase, deoxyribonuclease, and protease negative. Differentiation from other pathogenic coryneforms has been historically based on a lack of ability to ferment carbohydrates or liquefy gelatin.[2] Because it is sometimes acid fast, it may be mistaken for a *Mycobacterium.*[256] It can be distinguished from some mycobacterial species by the 14-day arylsulfatase test because *Rhodococcus* is negative for this reaction. Two special features of *R. equi* help distinguish it from other similar organisms: (1) When *R. equi* is cultured on sheep blood agar that is cross-streaked with other bacteria, such as *S. aureus, C. pseudotuberculosis,* or *Listeria monocytogenes,* synergistic hemolysis occurs (the CAMP test); and (2) in vitro antagonism between imipenem and other β-lactams is widespread among *R. equi* isolates.[267] In general, the identification of *Rhodococcus* spp. with traditional phenotypic and biochemical tests may be difficult and is unreliable. Molecular techniques, especially gene sequencing, are currently the only methods that provide definitive identification of most aerobic actinomyces, such as *Rhodococcus, Gordonia,* and *Tsukamurella.* The 16S rRNA sequencing method provides a rapid and accurate identification of *Rhodococcus* spp. and other aerobic actinomycetes, including *Gordonia* and *Tsukamurella.*[268,269] MALDI-TOF offers promise for the rapid identification of *Rhodococcus.*[270]

Pathogenicity

R. equi is a facultative, intracellular pathogen. It infects macrophages and survives inside the lysosomes. After phagocytosis by macrophages, *R. equi* arrests phagosomal maturation.[271] Its ability to cause chronic infection may be based on its complex cell wall, which is thought to prevent phagosome-lysosome fusion, resists the oxidative burst, and causes a nonspecific degranulation of lysosomes, which permits intrahistiocytic survival.[272] *R. equi* can also survive within human alveolar epithelial cells.[273] Virulence factors associated with *R. equi* infections in animals and in humans have been defined and include the plasmid-encoded antigens VapA and VapB, although they do not always appear to be necessary for infection in humans.[274,275] Histopathologic evaluation usually reveals a necrotizing granulomatous reaction. Endobronchial granulomas have been reported.[276] Multiple microabscesses may be seen. Malakoplakia is a rare, chronic, granulomatous inflammatory process that is associated with an impaired ability to process microorganisms within histiocytes. It is characterized by accumulations of benign macrophages associated with intracellular and extracellular aggregates of periodic acid–Schiff stain–positive histiocytes that contain lamellated iron and calcium inclusions and are termed *Michaelis-Guttman bodies.*[272] Lung malakoplakia is a rare condition; most of the reported patients had *R. equi* pneumonia.[277]

Clinical Manifestations

R. equi has been cultured from a variety of human tissues and fluids, including sputum, bronchial washings, lung tissue, pleural fluid, blood, heart valves, CSF, brain, skin, lymph nodes, peritoneal fluid, bone, stool, pharyngeal exudates, and wounds.[257,260,278–291] It has been recovered from peritoneal dialysate, intravenous catheters, and CSF after ventriculoperitoneal shunt insertion.[260,292,293] It produces a biofilm on intravascular catheters.[294] Pneumonia accounts for about 80% of human cases of infection reported in the literature.[259,295] Most published cases of pulmonary infection have occurred in immunocompromised hosts.[295] The lung was the only site of infection in more than 80% of cases; a concurrent extrapulmonary site was reported in about 20% of cases of pulmonary infection.[260] Typically, the presentation is subacute in onset. Common symptoms include fever, productive or nonproductive cough, and fatigue.[260] Pleuritic chest pain is also common. Hemoptysis has been reported in about 15% of patients. *R. equi* bacteremia frequently complicates pneumonia. Other complications include the development of lung abscess, pleural effusion, empyema, pneumothorax, endobronchial lesions, pericarditis, cardiac tamponade, and mediastinitis.[260,289,295,296] Chest radiographs reveal nodules, cavities (single or multiple), infiltrates, and pleural effusions.[265,289] More than one type of lesion may be present. In a case series of pulmonary cavitary lesions in HIV-infected persons, *R. equi* was the fifth most common microbiologically proven cause, accounting for about 9% of cases.[297] It followed *Mycobacterium tuberculosis, Pneumocystis jirovecii, Pseudomonas aeruginosa*, and *S. aureus* in frequency. The cavities have been described as thick walled and sometimes have an air-fluid level.[260] Necrotizing pneumonia caused by *R. equi* closely resembles tuberculosis or nocardiosis.[298] Nodules or cavities of the upper lung lobes, or both, may be seen. Air-fluid levels are seen in cavitary lesions caused by *R. equi* but not in those seen with tuberculosis. Mediastinal enlargement has been noted. The most common computed tomographic finding is consolidation with cavitation.[299] Although a good-quality sputum specimen can yield a microbiologic diagnosis, in many instances invasive techniques such as bronchoscopy, thoracentesis, or surgical resection are required in order to make a microbiologic diagnosis. Blood cultures are positive in about 50% of HIV-infected individuals and in 25% of solid-organ transplant recipients who are infected with *R. equi.* Up to 30% of immunocompetent hosts are bacteremic.[262,278,280]

Extrapulmonary infection with *R. equi* occurs in about 20% of cases with pulmonary infection; infection of extrapulmonary sites occurs in about 25% of cases without evidence of pulmonary involvement. The most common extrapulmonary sites reported were brain and subcutaneous abscesses.[260,300,301] Extrapulmonary infection is frequently a late manifestation of the initial pulmonary infection. Abscesses in the liver, spleen, thyroid, kidney, psoas muscle, bone, prostate, intraabdominal cavity, and paraspinous tissue have occurred.[295,300,302] Extrapulmonary infections not associated with pulmonary disease have been noted to manifest in three distinct patterns.[260] The first pattern includes wound infections, traumatic septic arthritis, and endophthalmitis after ocular injury. In these cases, infection remains localized at the primary site, and drainage procedures appear to hasten recovery. The second group consists of cases of isolated bacteremia that manifested with fever. Most of these patients had malignancies and were neutropenic or had recently received chemotherapy. Central venous catheters were present in most of these cases. The third pattern may have resulted from inoculation of the gastrointestinal tract with dissemination to regional lymph nodes. Conditions in this group include peritonitis, pelvic masses, and mesenteric adenitis. Other reported types of infection include otitis media with mastoiditis; colonic polyps infiltrated with *R. equi*; and osteomyelitis of the vertebrae, long bones, and mandible.[260,302]

More than 85% of cases of *R. equi* infection described in the literature have occurred in immunocompromised hosts, particularly those with HIV infection. HIV-infected patients account for two-thirds of cases.[259] Other immunocompromised hosts reported to be infected with *R. equi* include recipients of solid organ and hematopoietic stem cell transplants; diabetics; alcoholics; those with chronic renal failure, leukemia, lymphomas, lung cancer, or sarcoidosis; and preterm infants. Infection has occurred as a complication of chemotherapy, corticosteroid use, and treatment with monoclonal antibodies.[303,304] Immune reconstitution syndrome (IRIS) secondary to *R. equi* has been reported in a patient with HIV and Burkitt lymphoma.[305] Infection of immunocompetent persons with *R. equi,* however, may be more common than previously assumed because in a recent series, immunocompetent hosts accounted

for 42% of cases.[262] Clearance of *R. equi* is impaired in the immunocompromised host, and relapses are common despite maintenance antibiotic therapy.[306] In the pre–antiretroviral therapy era, relapses of pneumonia were described in up to 80% of HIV-infected patients. Infection occurs primarily in patients with CD4 counts of less than 100 cells/μL.[259] About 10% of *R. equi* infections occur in transplant recipients receiving immunosuppressive therapy and are generally a late complication.[259,282,288,306–309] Most of these patients were solid-organ transplant recipients. The primary site of infection was the lung. Findings included both nodular lesions and infiltrates. Cavitary lesions were frequent. Pseudotumor has been reported.[308] In about half of transplant recipients, extrapulmonary infection was present and included brain abscesses, paravertebral abscess, purulent pericarditis, subcutaneous nodules, and osteomyelitis of the femur. Among immunocompetent hosts, localized infections account for nearly 50%.[262] Pulmonary infection was present in more than 40%. Disseminated infection also occurred.[286,287,300] Recurrent infection has been reported.[310] The mortality rate is greatest among patients with AIDS and has been reported to be as high as 58%.[260,278,280] In a study from Thailand, HIV-infected persons with community-acquired pneumonia caused by *R. equi* were more likely to die than those infected with other organisms.[311] The use of highly active antiretroviral therapy has greatly improved the survival rates to 90% to 100% for those with HIV infection.[312,313] Mortality in immunocompetent hosts has been reported to be 11%; it is about 20% for non–HIV-infected immunocompromised hosts.[259,282]

Treatment

Susceptibility testing should be performed, based on CLSI recommendations, with a regular gram-positive panel. *R. equi* is usually susceptible in vitro to vancomycin, macrolides, fluoroquinolones, rifampin, teicoplanin, carbapenems, aminoglycosides, and linezolid.[259,260,314–316] Of the quinolones, moxifloxacin and gatifloxacin are the most active in vitro.[317,318] Gatifloxacin is no longer available in the United States. Susceptibility to clindamycin, tetracycline, chloramphenicol, and cephalosporins is variable; *R. equi* is usually resistant to penicillins, and even if susceptible in vitro, the use of penicillins and other β-lactams (except carbapenems) is not recommended because resistance can develop.[260,262] Mechanisms of resistance include altered penicillin-binding proteins and β-lactamase production.[262,298] In an animal model, the most effective agents were vancomycin, imipenem, and rifampin.[319] Rifampin, erythromycin, clarithromycin, vancomycin, and doxycycline exhibit a relatively long postantibiotic effect.[320] Rifampin-resistant isolates have been reported.[321] The incidence of resistance to macrolides and rifampin in animal isolates has been increasing.[322] Monotherapy has been ineffective in a number of cases and is not recommended. Monotherapy can lead to ciprofloxacin-resistant mutants.[323] Combinations of two or three antimicrobial agents have generally yielded partial or complete therapeutic responses. Localized, non-CNS infections in immunocompetent hosts can usually be treated with oral agents.[262] Two-drug regimens that include a macrolide, rifampin, fluoroquinolone, or a combination can be started empirically and should be adjusted based on the results of susceptibility testing.[259] Immunocompromised hosts and those with serious infections should be treated with two- or three-drug regimens that include vancomycin or a carbapenem (imipenem, ertapenem, or meropenem), rifampin, a fluoroquinolone, an aminoglycoside, or a macrolide.[309,324] Linezolid has been used successfully to treat relapsing infection and in cases of osteomyelitis.[306] It has been suggested that intravenous antibiotics be continued until clinical improvement occurs or for a minimum of 2 to 3 weeks.[259] Oral agents should then be given until cultures are negative and signs and symptoms have resolved. A 2- to 8-week course of two drugs might be sufficient for mild-to-moderate pneumonia, although optimal treatment of this infection is still unclear.[298] A minimum of 2 to 6 months of antimicrobial therapy is advised for immunocompromised hosts and those with pulmonary, bone, joint, or CNS infections. Brain abscess has been successfully treated with 8 weeks of intravenous therapy.[300] Because the CNS is a frequent secondary site of infection, agents that penetrate this site should be administered.[260] Drainage or débridement of localized abscesses, empyema, infected pericardial effusion, and large cavities may be beneficial.[325,326] Lung lobectomy has been performed when poor clinical response was noted with antimicrobial therapy.[327] It is generally recommended that after the treatment course is completed, HIV-infected individuals and persons with ongoing immunosuppression receive long-term suppressive therapy with a macrolide plus rifampin or a quinolone or doxycycline with rifampin. For HIV-positive patients, oral suppressive therapy should be continued until immune reconstitution occurs. Reducing immunosuppressive therapies may improve eradication of *Rhodococcus* spp.[308] Infection may develop or may manifest at other sites during therapy. Relapses are common. They can occur at the initial site of infection or at other sites.

Other *Rhodococcus* Species and Related Genera

Infections caused by other *Rhodococcus* spp. and related genera, such as *Gordonia* and *Tsukamurella,* have generally been associated with medical procedures or devices. *Gordonia* spp., previously classified as *Rhodococcus* spp., have caused pulmonary infections, bacteremia, endocarditis, septic arthritis, and CNS infections in both immunocompromised and immunocompetent adults and children.[328–338] Major pathogens include *Gordonia bronchialis, Gordonia sputi,* and *Gordonia terrae. G. terrae* has caused CNS infections, central venous catheter–associated bacteremia and endocarditis in children, and infections of medical devices including peritoneal dialysis catheters.[329, 330, 333,336] *G. sputi* has caused skin infections, mediastinitis after coronary bypass surgery, and bacteremia.[335, 337] *G. bronchialis* (formerly *Rhodococcus bronchialis*) has been reported to cause a cluster of sternal wound infections after coronary artery bypass surgery, bacteremia, osteomyelitis, pleural infection, and recurrent breast abscess.[339–345] Pulmonary infection resembling tuberculosis has been reported to be caused by *Gordonia rubropertincta* (formerly *Rhodococcus rubropertinctus*) in a patient who was not immunosuppressed.[346] Other human pathogens include *Gordonia polyisoprenivorans, Gordonia amicalis, Gordonia araii, Gordonia effusa, Gordonia otitidis,* and *Gordonia aichiensis.* They cause predominately skin infection, pulmonary infection, and bacteremia.[337,338] Speciation is best accomplished by 16S rRNA sequencing.[333] MALDI-TOF mass spectrometry has been used successfully to identify *G. bronchialis,* but other systems have been ineffective because of current database limitations.[270,342] *Gordonia* isolates are most predictably susceptible to imipenem, ciprofloxacin, amikacin, linezolid, gatifloxacin, and gentamicin. Eighty-nine percent of isolates were susceptible to vancomycin.[333] Initial treatment may consist of a carbapenem or a fluoroquinolone with or without an aminoglycoside.

Tsukamurella spp., including *Tsukamurella paurometabola* (formerly *Rhodococcus aurantiacus* and *Corynebacterium paurometabolum*), have caused bacteremia associated with central venous catheters in patients with malignancies and patients who were receiving parenteral nutrition, and in those with infection of an implantable cardioverter defibrillator, pneumonia (including tuberculosis-like pneumonia), meningitis, conjunctivitis, keratitis, otitis media, skin and soft tissue abscesses, brain abscess, peritonitis resulting from CAPD, and necrotizing tenosynovitis.[298,346–356] Underreporting of cases has probably occurred as a result of misidentification as atypical *Mycobacterium* spp. or as *Rhodococcus* spp.[298,356,357] Susceptibility varies with species. Most isolates have been susceptible to fluoroquinolones, macrolides, imipenem, vancomycin, trimethoprim-sulfamethoxazole, and aminoglycosides and resistant to penicillins and cephalosporins. Topical tetracycline or levofloxacin has been used to treat ocular and ear infections.

R. fascians (R. luteus) and *R. erythropolis* have been associated with chronic endophthalmitis after lens implantation.[358] *R. erythropolis* has been isolated from patients with peritonitis who were undergoing ambulatory peritoneal dialysis, from subcutaneous nodules in a patient with AIDS, from blood, and from sputum in a patient with pneumonia.[359–361] *R. rhodochrous* has caused pneumonia, bacteremia, pericarditis, skin lesions, meningoencephalitis, ventriculoperitoneal shunt infection, and chronic corneal ulceration.[362,363] *Rhodococcus globerulus* bacteremia was associated with a subacute illness with severe hepatitis in a hematopoietic stem cell transplant recipient. It also was associated with a corneal abscess after LASIK (laser-assisted in situ keratomileusis) therapy.[364,365] Meningitis caused by non-*equi Rhodococcus* has been reported in an immunocompetent host.[366] Antimicrobial therapy for these infections should be based on susceptibility testing. When *Rhodococcus* spp. infection occurs in association with a medical device, the device should be removed.

Key References

The complete reference list is available online at Expert Consult.

2. Funke G, von Graevenitz A, Clarridge JE 3rd, et al. Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev*. 1997;10:125–159.
3. Funke G, Bernard KA. Coryneform gram-positive rods. In: Jorgensen J, Pfaller MA, Carroll KC, et al, eds. *Manual of Clinical Microbiology*. Vol. 1. 11th ed. Washington, DC: American Society for Microbiology Press; 2015:474–503.
6. Chandran R, Puthukkichal DR, Suman E, et al. Diphtheroids-important nosocomial pathogens. *J Clin Diagn Res*. 2016;10:DC28–DC31.
8. Bernard KA, Munro C, Wiebe D, et al. Characteristics of rare or recently described *Corynebacterium* species recovered from human clinical material in Canada. *J Clin Microbiol*. 2002;40:4375–4381.
15. Bernard KA, Funke G. Genus *Corynebacterium*. In: Goodfellow M, Kampfer P, Busse H-J, et al, eds. *Bergey's Manual of Systemic Bacteriology*. Vol. 5. 2 ed. New York: Springer; 2012:245–289.
16. Bernard K. The genus *Corynebacterium* and other medically relevant coryneform-like bacteria. *J Clin Microbiol*. 2012;50:3152–3158.
18. Khamis A, Raoult D, La Scola B. rpoB gene sequencing for identification of *Corynebacterium* species. *J Clin Microbiol*. 2004;42:3925–3931.
19. Alatoom AA, Cazanave CJ, Cunningham SA, et al. Identification of non-diphtheriae *Corynebacterium* by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol*. 2012;50:160–163.
20. Soto A, Zapardiel J, Soriano F. Evaluation of API Coryne system for identifying coryneform bacteria. *J Clin Pathol*. 1994;47:756–759.
21. Funke G, Renaud FN, Freney J, et al. Multicenter evaluation of the updated and extended API (RAPID) Coryne database 2.0. *J Clin Microbiol*. 1997;35: 3122–3126.
22. Funke G, Peters K, Aravena-Roman M. Evaluation of the RapID CB plus system for identification of coryneform bacteria and *Listeria* spp. *J Clin Microbiol*. 1998;36:2439–2442.
23. Suwantarat N, Weik C, Romagnoli M, et al. Practical utility and accuracy of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of *Corynebacterium* species and other medically relevant coryneform-like bacteria. *Am J Clin Pathol*. 2016;145:22–28.
24. CLIS. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
30. Rajamani Sekar SK, Veeraraghavan B, Anandan S, et al. Strengthening the laboratory diagnosis of pathogenic *Corynebacterium* species in the vaccine era. *Lett Appl Microbiol*. 2017;65:354–365.
31. Zakikhany K, Efstratiou A. Diphtheria in Europe: current problems and new challenges. *Future Microbiol*. 2012;7:595–607.
32. Wagner KS, White JM, Crowcroft NS, et al. Diphtheria in the United Kingdom, 1986-2008: the increasing role of *Corynebacterium ulcerans*. *Epidemiol Infect*. 2010;138:1519–1530.
33. Both L, Collins S, de Zoysa A, et al. Molecular and epidemiological review of toxigenic diphtheria infections in England between 2007 and 2013. *J Clin Microbiol*. 2015;53:567–572.
34. Wagner KS, White JM, Lucenko I, et al. Diphtheria in the postepidemic period, Europe, 2000-2009. *Emerg Infect Dis*. 2012;18:217–225.
38. Efstratiou A, Engler KH, Mazurova IK, et al. Current approaches to the laboratory diagnosis of diphtheria. *J Infect Dis*. 2000;181(suppl 1):S138–S145.
55. Leal SM Jr, Jones M, Gilligan PH. Clinical significance of commensal gram-positive rods routinely isolated from patient samples. *J Clin Microbiol*. 2016;54:2928–2936.
56. Kimura SI, Gomyo A, Hayakawa J, et al. Clinical characteristics and predictive factors for mortality in coryneform bacteria bloodstream infection in hematological patients. *J Infect Chemother*. 2017;23:148–153.
57. Renom F, Gomila M, Garau M, et al. Respiratory infection by *Corynebacterium striatum*: epidemiological and clinical determinants. *New Microbes New Infect*. 2014;2:106–114.
72. McMullen AR, Anderson N, Wallace MA, et al. When good bugs go bad: epidemiology and antimicrobial resistance profiles of *Corynebacterium striatum*, an emerging multidrug-resistant, opportunistic pathogen. *Antimicrob Agents Chemother*. 2017;61:e01111-01117.
73. Hahn WO, Werth BJ, Butler-Wu SM, et al. Multidrug-resistant *Corynebacterium striatum* associated with increased use of parenteral antimicrobial drugs. *Emerg Infect Dis*. 2016;22:1908–1914.
91. Goldstein EJ, Citron DM, Merriam CV, et al. In vitro activities of the new semisynthetic glycopeptide telavancin (TD-6424), vancomycin, daptomycin, linezolid, and four comparator agents against anaerobic gram-positive species and *Corynebacterium* spp. *Antimicrob Agents Chemother*. 2004;48:2149–2152.
93. Fernandez-Roblas R, Adames H, Martin-de-Hijas NZ, et al. In vitro activity of tigecycline and 10 other antimicrobials against clinical isolates of the genus *Corynebacterium*. *Int J Antimicrob Agents*. 2009;33: 453–455.
117. Holzmann D, Funke G, Linder T, et al. *Turicella otitidis* and *Corynebacterium auris* do not cause otitis media with effusion in children. *Pediatr Infect Dis J*. 2002;21: 1124–1126.
137. Mookadam F, Cikes M, Baddour LM, et al. *Corynebacterium jeikeium* endocarditis: a systematic overview spanning four decades. *Eur J Clin Microbiol Infect Dis*. 2006;25:349–353.
152. Soriano F, Tauch A. Microbiological and clinical features of *Corynebacterium urealyticum*: urinary tract stones and genomics as the Rosetta Stone. *Clin Microbiol Infect*. 2008;14:632–643.
168. Dobinson HC, Anderson TP, Chambers ST, et al. Antimicrobial treatment options for granulomatous mastitis caused by *Corynebacterium* species. *J Clin Microbiol*. 2015;53:2895–2899.
216. Gomez-Garces JL, Alos JI, Tamayo J. In vitro activity of linezolid and 12 other antimicrobials against coryneform bacteria. *Int J Antimicrob Agents*. 2007;29:688–692.
235. Ramanan P, Barreto JN, Osmon DR, et al. *Rothia* bacteremia: a 10-year experience at Mayo Clinic, Rochester, Minnesota. *J Clin Microbiol*. 2014;52:3184–3189.
257. Yamshchikov AV, Schuetz A, Lyon GM. *Rhodococcus equi* infection. *Lancet Infect Dis*. 2010;10:350–359.
258. Topino S, Galati V, Grilli E, et al. *Rhodococcus equi* infection in HIV-infected individuals: case reports and review of the literature. *AIDS Patient Care STDS*. 2010;24:211–222.
263. Herath S, Lewis C, Nisbet M. Increasing awareness of *Rhodococcus equi* pulmonary infection in the immunocompetent adult: a rare infection with poor prognosis. *N Z Med J*. 2013;126:165–174.
268. Wang T, Kong F, Chen S, et al. Improved identification of *Gordonia*, *Rhodococcus* and *Tsukamurella* species by 5'-end 16S rRNA gene sequencing. *Pathology*. 2011;43:58–63.
289. Torres-Tortosa M, Arrizabalaga J, Villanueva JL, et al. Prognosis and clinical evaluation of infection caused by *Rhodococcus equi* in HIV-infected patients: a multicenter study of 67 cases. *Chest*. 2003;123:1970–1976.
294. Al Akhrass F, Al Wohoush I, Chaftari AM, et al. *Rhodococcus* bacteremia in cancer patients is mostly catheter related and associated with biofilm formation. *PLoS ONE*. 2012;7:e32945.
295. Cornish N, Washington JA. *Rhodococcus equi* infections: clinical features and laboratory diagnosis. *Curr Clin Top Infect Dis*. 1999;19:198–215.
298. Savini V, Fazii P, Favaro M, et al. Tuberculosis-like pneumonias by the aerobic actinomycetes *Rhodococcus*, *Tsukamurella* and *Gordonia*. *Microbes Infect*. 2012;14:401–410.
307. Menon V, Gottlieb T, Gallagher M, et al. Persistent *Rhodococcus equi* infection in a renal transplant patient: case report and review of the literature. *Transpl Infect Dis*. 2012;14:E126–E133.
313. Gundelly P, Suzuki Y, Ribes JA, et al. Differences in *Rhodococcus equi* infections based on immune status and antibiotic susceptibility of clinical isolates in a case series of 12 patients and cases in the literature. *Biomed Res Int*. 2016;2016:2737295.
315. Jacks SS, Giguere S, Nguyen A. In vitro susceptibilities of *Rhodococcus equi* and other common equine pathogens to azithromycin, clarithromycin, and 20 other antimicrobials. *Antimicrob Agents Chemother*. 2003;47:1742–1745.
322. Giguere S, Berghaus LJ, Willingham-Lane JM. Antimicrobial resistance in *Rhodococcus equi*. *Microbiol Spectr*. 2017;5.
333. Blaschke AJ, Bender J, Byington CL, et al. *Gordonia* species: emerging pathogens in pediatric patients that are identified by 16S ribosomal RNA gene sequencing. *Clin Infect Dis*. 2007;45:483–486.
338. Drzyzga O. The strengths and weaknesses of *Gordonia*: a review of an emerging genus with increasing biotechnological potential. *Crit Rev Microbiol*. 2012;38:300–316.
344. Johnson JA, Onderdonk AB, Cosimi LA, et al. *Gordonia bronchialis* bacteremia and pleural infection: case report and review of the literature. *J Clin Microbiol*. 2011;49:1662–1666.
355. Liu CY, Lai CC, Lee MR, et al. Clinical characteristics of infections caused by *Tsukamurella* spp. and antimicrobial susceptibilities of the isolates. *Int J Antimicrob Agents*. 2011;38:534–537.

References

1. Lehmann KB, Neumann R. *Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik*. Munich: JF Lehmann; 1896.
2. Funke G, von Graevenitz A, Clarridge JE 3rd, et al. Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev*. 1997;10:125–159.
3. Funke G, Bernard KA. Coryneform gram-positive rods. In: Jorgensen J, Pfaller MA, Carroll KC, et al, eds. *Manual of Clinical Microbiology*. Vol. 1. 11th ed. Washington, DC: American Society for Microbiology Press; 2015:474–503.
4. Grice EA, Kong HH, Conlan S, et al. Topographical and temporal diversity of the human skin microbiome. *Science*. 2009;324:1190–1192.
5. Stroman DW, Roland PS, Dohar J, et al. Microbiology of normal external auditory canal. *Laryngoscope*. 2001;111(11 Pt 1):2054–2059.
6. Chandran R, Puthukkichal DR, Suman E, et al. Diphtheroids-important nosocomial pathogens. *J Clin Diagn Res*. 2016;10:DC28–DC31.
7. Lagrou K, Verhaegen J, Janssens M, et al. Prospective study of catalase-positive coryneform organisms in clinical specimens: identification, clinical relevance, and antibiotic susceptibility. *Diagn Microbiol Infect Dis*. 1998;30:7–15.
8. Bernard KA, Munro C, Wiebe D, et al. Characteristics of rare or recently described *Corynebacterium* species recovered from human clinical material in Canada. *J Clin Microbiol*. 2002;40:4375–4381.
9. Belmares J, Detterline S, Pak JB, et al. *Corynebacterium* endocarditis species-specific risk factors and outcomes. *BMC Infect Dis*. 2007;7:4.
10. Blaise G, Nikkels AF, Hermanns-Le T, et al. *Corynebacterium*-associated skin infections. *Int J Dermatol*. 2008;47:884–890.
11. Barraclough K, Hawley CM, McDonald SP, et al. *Corynebacterium* peritonitis in Australian peritoneal dialysis patients: predictors, treatment and outcomes in 82 cases. *Nephrol Dial Transplant*. 2009;24:3834–3839.
12. Cazanave C, Greenwood-Quaintance KE, Hanssen AD, et al. *Corynebacterium* prosthetic joint infection. *J Clin Microbiol*. 2012;50:1518–1523.
13. Riegel P, Ruimy R, Christen R, et al. Species identities and antimicrobial susceptibilities of corynebacteria isolated from various clinical sources. *Eur J Clin Microbiol Infect Dise*. 1996;15:657–662.
14. Hollis DG, Weaver RE. Gram-Positive Organisms: A Guide to Identification. Atlanta: Centers for Disease Control and Prevention, CDC Special Bacteriology Section; 1981.
15. Bernard KA, Funke G. Genus *Corynebacterium*. In: Goodfellow M, Kampfer P, Busse H-J, et al, eds. *Bergey's Manual of Systemic Bacteriology*. Vol. 5. 2nd ed. New York: Springer; 2012:245–289.
16. Bernard K. The genus *Corynebacterium* and other medically relevant coryneform-like bacteria. *J Clin Microbiol*. 2012;50:3152–3158.
17. Tang YW, Von Graevenitz A, Waddington MG, et al. Identification of coryneform bacterial isolates by ribosomal DNA sequence analysis. *J Clin Microbiol*. 2000;38:1676–1678.
18. Khamis A, Raoult D, La Scola B. rpoB gene sequencing for identification of *Corynebacterium* species. *J Clin Microbiol*. 2004;42:3925–3931.
19. Alatoom AA, Cazanave CJ, Cunningham SA, et al. Identification of non-diphtheriae *Corynebacterium* by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol*. 2012;50:160–163.
20. Soto A, Zapardiel J, Soriano F. Evaluation of API Coryne system for identifying coryneform bacteria. *J Clin Pathol*. 1994;47:756–759.
21. Funke G, Renaud FN, Freney J, et al. Multicenter evaluation of the updated and extended API (RAPID) Coryne database 2.0. *J Clin Microbiol*. 1997;35:3122–3126.
22. Funke G, Peters K, Aravena-Roman M. Evaluation of the RapID CB plus system for identification of coryneform bacteria and *Listeria* spp. *J Clin Microbiol*. 1998;36:2439–2442.
23. Suwantarat N, Weik C, Romagnoli M, et al. Practical utility and accuracy of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of *Corynebacterium* species and other medically relevant coryneform-like bacteria. *Am J Clin Pathol*. 2016;145:22–28.
24. CLIS. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
25. Mendes RE, Sader HS, Flamm RK, et al. Activity of oritavancin tested against uncommonly isolated gram-positive pathogens responsible for documented infections in hospitals worldwide. *J Antimicrob Chemother*. 2014;69:1579–1581.
26. Mendes RE, Sader HS, Flamm RK, et al. Telavancin activity when tested by a revised susceptibility testing method against uncommonly isolated gram-positive pathogens responsible for documented infections in hospitals worldwide (2011-2013). *J Glob Antimicrob Resist*. 2015;3:36–39.
27. Olender A. Antibiotic resistance and detection of the most common mechanism of resistance (MLSB) of opportunistic *Corynebacterium*. *Chemotherapy*. 2013;59:294–306.
28. Power EG, Abdulla YH, Talsania HG, et al. vanA genes in vancomycin-resistant clinical isolates of *Oerskovia turbata* and *Arcanobacterium (Corynebacterium) haemolyticum*. *J Antimicrob Chemother*. 1995;36:595–606.
29. Wauters G, Van Bosterhaut B, Janssens M, et al. Identification of *Corynebacterium amycolatum* and other nonlipophilic fermentative corynebacteria of human origin. *J Clin Microbiol*. 1998;36:1430–1432.
30. Rajamani Sekar SK, Veeraraghavan B, Anandan S, et al. Strengthening the laboratory diagnosis of pathogenic *Corynebacterium* species in the vaccine era. *Lett Appl Microbiol*. 2017;65:354–365.
31. Zakikhany K, Efstratiou A. Diphtheria in Europe: current problems and new challenges. *Future Microbiol*. 2012;7:595–607.
32. Wagner KS, White JM, Crowcroft NS, et al. Diphtheria in the United Kingdom, 1986-2008: the increasing role of *Corynebacterium ulcerans*. *Epidemiol Infect*. 2010;138:1519–1530.
33. Both L, Collins S, de Zoysa A, et al. Molecular and epidemiological review of toxigenic diphtheria infections in England between 2007 and 2013. *J Clin Microbiol*. 2015;53:567–572.
34. Wagner KS, White JM, Lucenko I, et al. Diphtheria in the postepidemic period, Europe, 2000-2009. *Emerg Infect Dis*. 2012;18:217–225.
35. Bonmarin I, Guiso N, Le Fleche-Mateos A, et al. Diphtheria: a zoonotic disease in France? *Vaccine*. 2009;27:4196–4200.
36. Meinel DM, Konrad R, Berger A, et al. Zoonotic transmission of toxigenic *Corynebacterium ulcerans* strain, Germany, 2012. *Emerg Infect Dis*. 2015;21:356–358.
37. Konig C, Meinel DM, Margos G, et al. Multilocus sequence typing of *Corynebacterium ulcerans* provides evidence for zoonotic transmission and for increased prevalence of certain sequence types among toxigenic strains. *J Clin Microbiol*. 2014;52:4318–4324.
38. Efstratiou A, Engler KH, Mazurova IK, et al. Current approaches to the laboratory diagnosis of diphtheria. *J Infect Dis*. 2000;181(suppl 1):S138–S145.
39. De Zoysa A, Efstratiou A, Mann G, et al. Development, validation and implementation of a quadruplex real-time PCR assay for identification of potentially toxigenic corynebacteria. *J Med Microbiol*. 2016;65:1521–1527.
40. Wellinghausen N, Sing A, Kern WV, et al. A fatal case of necrotizing sinusitis due to toxigenic *Corynebacterium ulcerans*. *Int J Med Microbiol*. 2002;292:59–63.
41. Moore LSP, Leslie A, Meltzer M, et al. *Corynebacterium ulcerans* cutaneous diphtheria. *Lancet Infect Dis*. 2015;15:1100–1107.
42. Dessau RB, Brandtchristensen M, Jensen OJ, et al. Pulmonary nodules due to *Corynebacterium ulcerans*. *Eur Respir J*. 1995;8:651–653.
43. Nureki S, Miyazaki E, Matsuno O, et al. *Corynebacterium ulcerans* infection of the lung mimicking the histology of Churg-Strauss syndrome. *Chest*. 2007;131:1237–1239.
44. Konrad R, Hormansdorfer S, Sing A. Possible human-to-human transmission of toxigenic *Corynebacterium ulcerans*. *Clin Microbiol Infect*. 2015;21:768–771.
45. Peel MM, Palmer GG, Stacpoole AM, et al. Human lymphadenitis due to *Corynebacterium pseudotuberculosis*: report of ten cases from Australia and review. *Clin Infect Dis*. 1997;24:185–191.
46. Richards M, Hurse A. *Corynebacterium pseudotuberculosis* abscesses in a young butcher. *Aust N Z J Med*. 1985;15:85–86.
47. Heggelund L, Gaustad P, Havelsrud OE, et al. *Corynebacterium pseudotuberculosis* pneumonia in a veterinary student infected during laboratory work. *Open Forum Infect Dis*. 2015;2:ofv053.
48. Porschen RK, Goodman Z, Rafai B. Isolation of *Corynebacterium xerosis* from clinical specimens: infection and colonization. *Am J Clin Pathol*. 1977;68:290–293.
49. Esteban J, Nieto E, Calvo R, et al. Microbiological characterization and clinical significance of *Corynebacterium amycolatum* strains. *Eur J Clin Microbiol Infect Dis*. 1999;18:518–521.
50. Funke G, Lawson PA, Bernard KA, et al. Most *Corynebacterium xerosis* strains identified in the routine clinical laboratory correspond to *Corynebacterium amycolatum*. *J Clin Microbiol*. 1996;34:1124–1128.
51. Wooster SL, Qamruddin A, Clarke R, et al. Brain abscess due to *Corynebacterium xerosis*. *J Infect*. 1999;38:55–56.
52. Robins E, Haile-Selassie T. *Corynebacterium xerosis* sepsis in a pediatric patient with sickle cell disease (a case report). *Clin Pediatr (Phila)*. 2001;40:181–182.
53. Haque RM, Torkildsen GL, Brubaker K, et al. Multicenter open-label study evaluating the efficacy of azithromycin ophthalmic solution 1% on the signs and symptoms of subjects with blepharitis. *Cornea*. 2010;29:871–877.
54. Voisin S, Deruaz D, Freney J, et al. Differentiation of *Corynebacterium amycolatum*, *C. minutissimum*, *C. striatum* and related species by pyrolysis-gas-liquid chromatography with atomic emission detection. *Res Microbiol*. 2002;153:307–311.
55. Leal SM Jr, Jones M, Gilligan PH. Clinical significance of commensal gram-positive rods routinely isolated from patient samples. *J Clin Microbiol*. 2016;54:2928–2936.
56. Kimura SI, Gomyo A, Hayakawa J, et al. Clinical characteristics and predictive factors for mortality in coryneform bacteria bloodstream infection in hematological patients. *J Infect Chemother*. 2017;23:148–153.
57. Renom F, Gomila M, Garau M, et al. Respiratory infection by *Corynebacterium striatum*: epidemiological and clinical determinants. *New Microbes New Infect*. 2014;2:106–114.
58. Ishiwada N, Watanabe M, Murata S, et al. Clinical and bacteriological analyses of bacteremia due to *Corynebacterium striatum*. *J Infect Chemother*. 2016;22:790–793.
59. Boltin D, Katzir M, Bugoslavsky V, et al. *Corynebacterium striatum*–a classic pathogen eluding diagnosis. *Eur J Intern Med*. 2009;20:e49–e52.
60. Hong HL, Koh HI, Lee AJ. Native valve endocarditis due to *Corynebacterium striatum* confirmed by 16S ribosomal RNA sequencing: a case report and literature review. *J Infect Chemother*. 2016;48:239–245.
61. Yamamoto T, Kenzaka T, Mizuki S, et al. An extremely rare case of tubo-ovarian abscesses involving *Corynebacterium striatum* as causative agent. *BMC Infect Dis*. 2016;16:527.
62. Verma R, Kravitz GR. *Corynebacterium striatum* empyema and osteomyelitis in a patient with advanced rheumatoid arthritis. *BMJ Case Rep*. 2016;2016.
63. Westblade LF, Shams F, Duong S, et al. Septic arthritis of a native knee joint due to *Corynebacterium striatum*. *J Clin Microbiol*. 2014;52:1786–1788.
64. Mufty H, Smeets A, Christiaens MR. An atypical case of necrotizing fasciitis of the breast. *Acta Chir Belg*. 2014;114:215–218.
65. Hascoet S, Mauri L, Claude C, et al. Infective endocarditis risk after percutaneous pulmonary valve implantation with the Melody and Sapien valves. *JACC Cardiovasc Interv*. 2017;10:510–517.
66. Severo CB, Guazzelli LS, Barra MB, et al. Multiple pulmonary nodules caused by *Corynebacterium striatum* in an immunocompetent patient. *Rev Inst Med Trop Sao Paulo*. 2014;56:89–91.
67. Xu J, Yang Q, Li J, et al. The left atrial bacterial vegetative mass due to *Corynebacterium striatum* as a presentation of myxoma: a case report. *BMC Infect Dis*. 2017;17:368–373.
68. Verroken A, Bauraing C, Deplano A, et al. Epidemiological investigation of a nosocomial outbreak of multidrug-resistant *Corynebacterium striatum* at one Belgian university hospital. *Clin Microbiol Infect*. 2014;20:44–50.
69. Wang J, Wang Y, Du X, et al. Rapid transmission of multidrug-resistant *Corynebacterium striatum* among susceptible patients in a tertiary hospital in China. *J Infect Dev Ctries*. 2016;10:1299–1305.
70. Souza C, Faria YV, Sant'Anna Lde O, et al. Biofilm production by multiresistant *Corynebacterium striatum* associated with nosocomial outbreak. *Mem Inst Oswaldo Cruz*. 2015;110:242–248.
71. Renom F, Garau M, Rubi M, et al. Nosocomial outbreak of *Corynebacterium striatum* infection in patients with chronic obstructive pulmonary disease. *J Clin Microbiol*. 2007;45:2064–2067.
72. McMullen AR, Anderson N, Wallace MA, et al. When good bugs go bad: epidemiology and antimicrobial resistance profiles of *Corynebacterium striatum*, an emerging multidrug-resistant, opportunistic pathogen. *Antimicrob Agents Chemother*. 2017;61:e01111-01117.
73. Hahn WO, Werth BJ, Butler-Wu SM, et al. Multidrug-resistant *Corynebacterium striatum* associated with increased use of parenteral antimicrobial drugs. *Emerg Infect Dis*. 2016;22:1908–1914.

74. Qin L, Sakai Y, Bao R, et al. Characteristics of multidrug-resistant *Corynebacterium* spp. isolated from blood cultures of hospitalized patients in Japan. *Jpn J Infect Dis*. 2017;70:152–157.
75. Tran TT, Jaijakul S, Lewis CT, et al. Native valve endocarditis caused by *Corynebacterium striatum* with heterogeneous high-level daptomycin resistance: collateral damage from daptomycin therapy? *Antimicrob Agents Chemother*. 2012;56:3461–3464.
76. Werth BJ, Hahn WO, Butler-Wu SM, et al. Emergence of high-level daptomycin resistance in *Corynebacterium striatum* in two patients with left ventricular assist device infections. *Microb Drug Resist*. 2016;22:233–237.
77. McElvania TeKippe E, Thomas BS, Ewald GA, et al. Rapid emergence of daptomycin resistance in clinical isolates of *Corynebacterium striatum*—a cautionary tale. *Eur J Clin Microbiol Infect Dis*. 2014;33:2199–2205.
78. Yassin AF, Steiner U, Ludwig W. *Corynebacterium aurimucosum* sp. nov. and emended description of *Corynebacterium minutissimum* Collins and Jones (1983). *Int J Syst Evol Microbiol*. 2002;52(Pt 3):1001–1005.
79. Zinkernagel AS, von Graevenitz A, Funke G. Heterogeneity within *Corynebacterium minutissimum* strains is explained by misidentified *Corynebacterium amycolatum* strains. *Am J Clin Pathol*. 1996;106: 378–383.
80. Aperis G, Moyssakis I. *Corynebacterium minutissimum* endocarditis: a case report and review. *J Infect*. 2007;54:e79–e81.
81. Reece RM, Cunha CB, Rich JD. *Corynebacterium minutissimum* vascular graft infection: case report and review of 281 cases of prosthetic device-related *Corynebacterium* infection. *Scand J Infect Dis*. 2014;46:609–616.
82. Shin JY, Lee WK, Seo YH, et al. Postoperative abdominal infection caused by *Corynebacterium minutissimum*. *J Infect Chemother*. 2014;46:261–263.
83. Dalal A, Likhi R. *Corynebacterium minutissimum* bacteremia and meningitis: a case report and review of literature. *J Infect*. 2008;56:77–79.
84. Eshwara VK, Munim F, Shetty A, et al. *Corynebacterium minutissimum* infecting pseudomeningocele: a rare case. *J Microbiol Immunol Infect*. 2014;47:149–151.
85. Collins MD, Burton RA, Jones D. *Corynebacterium amycolatum* sp. nov. a new mycolic acid-less *Corynebacterium* species from human skin. *FEMS Microbiol*. 1988;49:349–352.
86. Clarke R, Qamruddin A, Taylor M, et al. Septic arthritis caused by *Corynebacterium amycolatum* following vascular graft sepsis. *J Infect*. 1999;38:126–127.
87. Daniels C, Schoors D, Van Camp G. Native valve endocarditis with aorta-to-left atrial fistula due to *Corynebacterium amycolatum*. *Eur J Echocardiogr*. 2003;4:68–70.
88. Adderson EE, Boudreaux JW, Hayden RT. Infections caused by coryneform bacteria in pediatric oncology patients. *Pediatr Infect Dis J*. 2008;27:136–141.
89. Sengupta M, Naina P, Balaji V, et al. *Corynebacterium amycolatum*: an unexpected pathogen in the ear. *J Clin Diagn Res*. 2015;9:DD1–DD3.
90. Toribio JA, Marrodan T, Fernandez-Natal I. Orbital implant infection by *Corynebacterium amycolatum*. *Orbit*. 2017;36:344–346.
91. Goldstein EJ, Citron DM, Merriam CV, et al. In vitro activities of the new semisynthetic glycopeptide telavancin (TD-6424), vancomycin, daptomycin, linezolid, and four comparator agents against anaerobic gram-positive species and *Corynebacterium* spp. *Antimicrob Agents Chemother*. 2004;48:2149–2152.
92. Funke G, Punter V, von Graevenitz A. Antimicrobial susceptibility patterns of some recently established coryneform bacteria. *Antimicrob Agents Chemother*. 1996;40:2874–2878.
93. Fernandez-Roblas R, Adames H, Martin-de-Hijas NZ, et al. In vitro activity of tigecycline and 10 other antimicrobials against clinical isolates of the genus *Corynebacterium*. *Int J Antimicrob Agents*. 2009;33:453–455.
94. Dalal A, Urban C, Segal-Maurer S. Endocarditis due to *Corynebacterium amycolatum*. *J Med Microbiol*. 2008;57:1299–1302.
95. Funke G, Bernard K, Bucher C. *Corynebacterium glucuronolyticum* sp. nov. isolated from male patients with genitourinary tract infections. *Med Microbiol Lett*. 1995;4:204–215.
96. Devriese LA, Riegel P, Hommez J, et al. Identification of *Corynebacterium glucuronolyticum* strains from the urogenital tract of humans and pigs. *J Clin Microbiol*. 2000;38:4657–4659.
97. Gherardi G, Di Bonaventura G, Pompilio A, et al. *Corynebacterium glucuronolyticum* causing genitourinary tract infection: case report and review of the literature. *IDCases*. 2015;2:56–58.
98. Curry CR, Saluja K, Das S, et al. Encrusted cystitis secondary to *Corynebacterium glucuronolyticum* in a 57-year-old man without predisposing factors. *Lab Med*. 2015;46:136–139.
99. Novo-Veleiro I, Hernandez-Cabrera M, Canas-Hernandez F, et al. Paucisymptomatic infectious prostatitis as a cause of fever without an apparent origin. A series of 19 patients. *Eur J Clin Microbiol Infect Dis*. 2013;32:263–268.
100. Kania RE, Lamers GE, Vonk MJ, et al. Characterization of mucosal biofilms on human adenoid tissues. *Laryngoscope*. 2008;118:128–134.
101. Funke G, Lawson PA, Collins MD. *Corynebacterium riegelii* sp. nov., an unusual species isolated from female patients with urinary tract infections. *J Clin Microbiol*. 1998;36:624–627.
102. Funke G, Osorio CR, Frei R, et al. *Corynebacterium confusum* sp. nov., isolated from human clinical specimens. *Int J Syst Bacteriol*. 1998;48(Pt 4):1291–1296.
103. Wattiau P, Janssens M, Wauters G. *Corynebacterium simulans* sp. nov., a non-lipophilic, fermentative *Corynebacterium*. *Int J Syst Evol Microbiol*. 2000;50(Pt 1):347–353.
104. Collins MD, Bernard KA, Hutson RA, et al. *Corynebacterium sundsvallense* sp. nov., from human clinical specimens. *Int J Syst Bacteriol*. 1999;49(Pt 2): 361–366.
105. Zimmermann O, Sproer C, Kroppenstedt RM, et al. *Corynebacterium thomssenii* sp. nov., a *Corynebacterium* with N-acetyl-beta-glucosaminidase activity from human clinical specimens. *Int J Syst Bacteriol*. 1998;48(Pt 2):489–494.
106. Renaud FN, Aubel D, Riegel P, et al. *Corynebacterium freneyi* sp. nov., alpha-glucosidase-positive strains related to *Corynebacterium xerosis*. *Int J Syst Evol Microbiol*. 2001;51(Pt 5):1723–1728.
107. Fernandez-Natal MI, Saez-Nieto JA, Fernandez-Roblas R, et al. The isolation of *Corynebacterium coyleae* from clinical samples: clinical and microbiological data. *Eur J Clin Microbiol Infect Dis*. 2008;27:177–184.
108. Riegel P, Creti R, Mattei R, et al. Isolation of *Corynebacterium tuscaniae* sp. nov. from blood cultures of a patient with endocarditis. *J Clin Microbiol*. 2006;44:307–312.
109. Funke G, Englert R, Frodl R, et al. *Corynebacterium canis* sp. nov., isolated from a wound infection caused by a dog bite. *Int J Syst Evol Microbiol*. 2010;60(Pt 11):2544–2547.
110. Iroh Tam PY, Fisher MA, Miller NS. *Corynebacterium falsenii* bacteremia occurring in an infant on vancomycin therapy. *J Clin Microbiol*. 2010;48:3440–3442.
111. Funke G, Frodl R, Bernard KA, et al. *Corynebacterium freiburgense* sp nov., isolated from a wound obtained from a dog bite. *Int J Syst Evol Microbiol*. 2009;59:2054–2057.
112. Aravena-Roman M, Sproer C, Straubler B, et al. *Corynebacterium pilbarense* sp. nov., a non-lipophilic *Corynebacterium* isolated from a human ankle aspirate. *Int J Syst Evol Microbiol*. 2010;60(Pt 7):1484–1487.
113. Bernard KA, Wiebe D, Burdz T, et al. Assignment of *Brevibacterium stationis* (ZoBell and Upham 1944) Breed 1953 to the genus *Corynebacterium*, as *Corynebacterium stationis* comb. nov., and emended description of the genus *Corynebacterium* to include isolates that can alkalinize citrate. *Int J Syst Evol Microbiol*. 2010;60:874–879.
114. Merhej V, Falsen E, Raoult D, et al. *Corynebacterium timonense* sp. nov. and *Corynebacterium massiliense* sp. nov., isolated from human blood and human articular hip fluid. *Int J Syst Evol Microbiol*. 2009;59(Pt 8):1953–1959.
115. Riegel P, de Briel D, Prevost G, et al. Taxonomic study of *Corynebacterium* group ANF-1 strains: proposal of *Corynebacterium afermentans* sp. nov. containing the subspecies *C. afermentans* subsp. *afermentans* subsp. nov. and *C. afermentans* subsp. *lipophilum* subsp. nov. *Int J Syst Bacteriol*. 1993;43:287–292.
116. Kumari P, Tyagi A, Marks P, et al. *Corynebacterium afermentans* spp. *afermentans* sepsis in a neurosurgical patient. *J Infect*. 1997;35:201–202.
117. Holzmann D, Funke G, Linder T, et al. *Turicella otitidis* and *Corynebacterium auris* do not cause otitis media with effusion in children. *Pediatr Infect Dis J*. 2002;21:1124–1126.
118. Morris A, Guild I. Endocarditis due to *Corynebacterium pseudodiphtheriticum*: five case reports, review, and antibiotic susceptibilities of nine strains. *Rev Infect Dis*. 1991;13:887–892.
119. Van Roeden SE, Thijsen SF, Sankatsing SU, et al. Clinical relevance of *Corynebacterium pseudodiphtheriticum* in lower respiratory tract specimens. *Infect Dis (Lond)*. 2015;47:862–868.
120. Diez-Aguilar M, Ruiz-Garbajosa P, Fernandez-Olmos A, et al. Non-diphtheriae *Corynebacterium* species: an emerging respiratory pathogen. *Eur J Clin Microbiol Infect Dis*. 2013;32:769–772.
121. Gutierrez-Rodero F, Ortiz de la Tabla V, Martinez C, et al. *Corynebacterium pseudodiphtheriticum*: an easily missed respiratory pathogen in HIV-infected patients. *Diagn Microbiol Infect Dis*. 1999;33:209–216.
122. Bittar F, Cassagne C, Bosdure E, et al. Outbreak of *Corynebacterium pseudodiphtheriticum* infection in cystic fibrosis patients, France. *Emerg Infect Dis*. 2010;16:1231–1236.
123. Camello TC, Souza MC, Martins CA, et al. *Corynebacterium pseudodiphtheriticum* isolated from relevant clinical sites of infection: a human pathogen overlooked in emerging countries. *Lett Appl Microbiol*. 2009;48:458–464.
124. Indumathi VA, Shikha R, Suryaprakash DR. Diphtheria-like illness in a fully immunised child caused by *Corynebacterium pseudodiphtheriticum*. *Indian J Med Microbiol*. 2014;32:443–445.
125. Roy S, Marla S, Praneetha DC. Recognition of *Corynebacterium pseudodiphtheriticum* by toll-like receptors and up-regulation of antimicrobial peptides in human corneal epithelial cells. *Virulence*. 2015;6:716–721.
126. Riegel P, Debriel D, Prevost G, et al. Proposal of *Corynebacterium propinquum* sp. nov. for *Corynebacterium* group ANF-3 Strains. *FEMS Microbiol*. 1993;113:229–234.
127. Kawasaki Y, Matsubara K, Ishihara H, et al. *Corynebacterium propinquum* as the first cause of infective endocarditis in childhood. *J Infect Chemother*. 2014;20:317–319.
128. Rea B, Hawkins J, Min H, et al. *Corynebacterium propinquum* endocarditis: a confounding presentation of a rare entity. *Cardiovasc Pathol*. 2017;28:71–73.
129. Todokoro D, Eguchi H, Yamada N, et al. Contact lens-related infectious keratitis with white plaque formation caused by *Corynebacterium propinquum*. *J Clin Microbiol*. 2015;53:3092–3095.
130. Abdolrasouli A, Roushan A. *Corynebacterium propinquum* associated with acute, nongonococcal urethritis. *Sex Transm Dis*. 2013;40:829–831.
131. Hande KR, Witebsky FG, Brown MS, et al. Sepsis with a new species of *Corynebacterium*. *Ann Intern Med*. 1976;85:423–426.
132. Jackman PJH, Pitcher DG, Pelczynska S, et al. Classification of corynebacteria associated with endocarditis (group JK) as *Corynebacterium jeikeium* sp. nov. *Syst Appl Microbiol*. 1987;9:83–90.
133. Salipante SJ, Sengupta DJ, Cummings LA, et al. Whole genome sequencing indicates *Corynebacterium jeikeium* compromises 4 separate genomospecies and identifies a dominant genomospecies among clinical isolates. *Int J Med Microbiol*. 2014;304:1001–1010.
134. Soriano F, Rodriguez-Tudela JL, Fernandez-Roblas R, et al. Skin colonization by *Corynebacterium* groups D2 and JK in hospitalized patients. *J Clin Microbiol*. 1988;26:1878–1880.
135. Young VM, Meyers WF, Moody MR, et al. The emergence of coryneform bacteria as a cause of nosocomial infections in compromised hosts. *Am J Med*. 1981;70:646–650.
136. van der Lelie H, Leverstein-Van Hall M, Mertens M, et al. *Corynebacterium* CDC group JK (*Corynebacterium jeikeium*) sepsis in haematological patients: a report of three cases and a systematic literature review. *Scand J Infect Dis*. 1995;27:581–584.
137. Mookadam F, Cikes M, Baddour LM, et al. *Corynebacterium jeikeium* endocarditis: a systematic overview spanning four decades. *Eur J Clin Microbiol Infect Dis*. 2006;25:349–353.
138. Greene KA, Clark RJ, Zabramski JM. Ventricular CSF shunt infections associated with *Corynebacterium jeikeium*: report of three cases and review. *Clin Infect Dis*. 1993;16:139–141.
139. Saritas T, Brandenburg V, Federico G, et al. Glomerulonephritis triggered by a chronically infected left ventricular assist device. *Lancet*. 2015;386:2363–2364.
140. Liu XL, Peng H, Mo TT, et al. Malignant otitis externa in a healthy non-diabetic patient. *Eur Arch Otorhinolaryngol*. 2016;273:2261–2265.
141. Dan M, Somer I, Knobel B, et al. Cutaneous manifestations of infection with *Corynebacterium* group JK. *Rev Infect Dis*. 1988;10:1204–1207.
142. Olson JM, Nguyen VQ, Yoo J, et al. Cutaneous manifestations of *Corynebacterium jeikeium* sepsis. *Int J Dermatol*. 2009;48:886–888.
143. Traub WH, Geipel U, Leonhard B, et al. Antibiotic susceptibility testing (agar disk diffusion and agar dilution) of clinical isolates of *Corynebacterium jeikeium*. *Chemotherapy*. 1998;44:230–237.
144. Rosato AE, Lee BS, Nash KA. Inducible macrolide resistance in *Corynebacterium jeikeium*. *Antimicrob Agents Chemother*. 2001;45:1982–1989.
145. Wang CC, Mattson D, Wald A. *Corynebacterium jeikeium* bacteremia in bone marrow transplant patients with

Hickman catheters. *Bone Marrow Transplant*. 2001;27:445–449.

146. Lappa A, Donfrancesco S, Picozzi P, et al. Treatment with daptomycin for *Corynebacterium jeikeium* left-sided prosthetic valve endocarditis. *Minerva Anestesiol*. 2012;78:729–732.
147. Dinleyici EC, Yargic ZA, Bor O, et al. Tigecycline treatment of multi-drug resistant *Corynebacterium jeikeium* infection in a child with relapsing and refractory acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2010;55:349–351.
148. Schoen C, Unzicker C, Stuhler G, et al. Life-threatening infection caused by daptomycin-resistant *Corynebacterium jeikeium* in a neutropenic patient. *J Clin Microbiol*. 2009;47:2328–2331.
149. Pitcher D, Soto A, Soriano F, et al. Classification of coryneform bacteria associated with human urinary tract infection (group D2) as *Corynebacterium urealyticum* sp. nov. *Int J Syst Bacteriol*. 1992;42:178–181.
150. Soriano F, Ponte C, Santamaria M, et al. *Corynebacterium* group D2 as a cause of alkaline-encrusted cystitis: report of four cases and characterization of the organisms. *J Clin Microbiol*. 1985;21:788–792.
151. Salem N, Salem L, Saber S, et al. *Corynebacterium urealyticum*: a comprehensive review of an understated organism. *Infect Drug Resist*. 2015;8:129–145.
152. Soriano F, Tauch A. Microbiological and clinical features of *Corynebacterium urealyticum*: urinary tract stones and genomics as the Rosetta Stone. *Clin Microbiol Infect*. 2008;14:632–643.
153. Lopez-Medrano F, Garcia-Bravo M, Morales JM, et al. Urinary tract infection due to *Corynebacterium urealyticum* in kidney transplant recipients: an underdiagnosed etiology for obstructive uropathy and graft dysfunction-results of a prospective cohort study. *Clin Infect Dis*. 2008;46:825–830.
154. Soriano F, Ponte C, Santamaria M, et al. In vitro and in vivo study of stone formation by *Corynebacterium* group D2 (*Corynebacterium urealyticum*). *J Clin Microbiol*. 1986;23:691–694.
155. Fernandez-Natal I, Guerra J, Alcoba M, et al. Bacteremia caused by multiply resistant *Corynebacterium urealyticum*: six case reports and review. *Eur J Clin Microbiol Infect Dis*. 2001;20:514–517.
156. Cappuccino L, Bottino P, Torricella A, et al. Nephrolithiasis by *Corynebacterium urealyticum* infection: literature review and case report. *J Nephrol*. 2014;27:117–125.
157. Meria P, Desgrippes A, Fournier R, et al. The conservative management of *Corynebacterium* group D2 encrusted pyelitis. *BJU Int*. 1999;84:270–275.
158. Neubauer M, Sourek J, Ryc M, et al. *Corynebacterium accolens* sp. nov., a gram-positive rod exhibiting satellitism, from clinical material. *Syst Appl Microbiol*. 1991;14:46–51.
159. Claeys G, Vanhouteghem H, Riegel P, et al. Endocarditis of native aortic and mitral valves due to *Corynebacterium accolens*: report of a case and application of phenotypic and genotypic techniques for identification. *J Clin Microbiol*. 1996;34:1290–1292.
160. Ang LM, Brown H. *Corynebacterium accolens* isolated from breast abscess: possible association with granulomatous mastitis. *J Clin Microbiol*. 2007;45:1666–1668.
161. Wong JS, Seaward LM, Ho CP, et al. *Corynebacterium accolens*-associated pelvic osteomyelitis. *J Clin Microbiol*. 2010;48:654–655.
162. Prevaes SM, de Winter-de Groot KM, Janssens HM, et al. Development of the nasopharyngeal microbiota in infants with cystic fibrosis. *Am J Respir Crit Care Med*. 2016;193:504–515.
163. Funke G, Pagano-Niederer M, Bernauer W. *Corynebacterium macginleyi* has to date been isolated exclusively from conjunctival swabs. *J Clin Microbiol*. 1998;36:3670–3673.
164. Ferreira CS, Figueira L, Moreira-Goncalves N, et al. Clinical and microbiological profile of bacterial microbial keratitis in a Portuguese tertiary referral center-where are we in 2015? *Eye Contact Lens*. 2018;44:15–20.
165. Villamil-Cajoto I, Rodriguez-Otero L, Garcia-Zabarte MA, et al. Septicemia caused by *Corynebacterium macginleyi*: a rare form of extraocular infection. *Int J Infect Dis*. 2008;12:333–335.
166. Kebbe J, Mador MJ. *Corynebacterium macginleyi*: a cause of ventilator associated pneumonia in an immunocompromised patient. *Respir Med Case Rep*. 2015;16:154–156.
167. Taylor GB, Paviour SD, Musaad S, et al. A clinicopathological review of 34 cases of inflammatory breast disease showing an association between corynebacteria infection and granulomatous mastitis. *Pathology*. 2003;35:109–119.
168. Dobinson HC, Anderson TP, Chambers ST, et al. Antimicrobial treatment options for granulomatous mastitis caused by *Corynebacterium* species. *J Clin Microbiol*. 2015;53:2895–2899.
169. Tauch A, Fernandez-Natal I, Soriano F. A microbiological and clinical review on *Corynebacterium kroppenstedtii*. *Int J Infect Dis*. 2016;48:33–39.
170. Paviour S, Musaad S, Roberts S, et al. *Corynebacterium* species isolated from patients with mastitis. *Clin Infect Dis*. 2002;35:1434–1440.
171. Johnstone KJ, Robson J, Cherian SG, et al. Cystic neutrophilic granulomatous mastitis associated with *Corynebacterium* including *Corynebacterium kroppenstedtii*. *Pathology*. 2017;49:405–412.
172. Bercot B, Kannengiesser C, Oudin C, et al. First description of NOD2 variant associated with defective neutrophil responses in a woman with granulomatous mastitis related to corynebacteria. *J Clin Microbiol*. 2009;47:3034–3037.
173. Fernandez-Natal I, Rodriguez-Lazaro D, Marrodan-Ciordia T, et al. Characterization and antimicrobial susceptibility of one antibiotic-sensitive and one multidrug-resistant *Corynebacterium kroppenstedtii* strain isolated from patients with granulomatous mastitis. *New Microbes New Infect*. 2016;14:93–97.
174. Hagemann JB, Essig A, Herrmann M, et al. Early prosthetic valve endocarditis caused by *Corynebacterium kroppenstedtii*. *Int J Med Microbiol*. 2015;305:957–959.
175. Hinic V, Lang C, Weisser M, et al. *Corynebacterium tuberculostearicum*: a potentially misidentified and multiresistant *Corynebacterium* species isolated from clinical specimens. *J Clin Microbiol*. 2012;50:2561–2567.
176. Sattar A, Yu S, Koirala J. *Corynebacterium* CDC group G native and prosthetic valve endocarditis. *Infect Dis Rep*. 2015;7:5881.
177. Vale JA, Scott GW. *Corynebacterium bovis* as a cause of human disease. *Lancet*. 1977;2:682–684.
178. Dalal A, Urban C, Ahluwalia M, et al. *Corynebacterium bovis* line related septicemia: a case report and review of the literature. *Scand J Infect Dis*. 2008;40:575–577.
179. Achermann Y, Trampuz A, Moro F, et al. *Corynebacterium bovis* shoulder prosthetic joint infection: the first reported case. *Diagn Microbiol Infect Dis*. 2009;64:213–215.
180. Riegel P, Ruimy R, de Briel D, et al. Genomic diversity and phylogenetic relationships among lipid-requiring diphtheroids from humans and characterization of *Corynebacterium macginleyi* sp. nov. *Int J Syst Bacteriol*. 1995;45:128–133.
181. Otsuka Y, Kawamura Y, Koyama T, et al. *Corynebacterium resistens* sp. nov., a new multidrug-resistant coryneform bacterium isolated from human infections. *J Clin Microbiol*. 2005;43:3713–3717.
182. Yassin AF. *Corynebacterium ureicelerivorans* sp. nov., a lipophilic bacterium isolated from blood culture. *Int J Syst Evol Microbiol*. 2007;57(Pt 6):1200–1203.
183. Fernandez-Natal MI, Saez-Nieto JA, Valdezate S, et al. Isolation of *Corynebacterium ureicelerivorans* from normally sterile sites in humans. *Eur J Clin Microbiol Infect Dis*. 2009;28:677–681.
184. Collins MD, Jones D, Schofield GM. Reclassification of '*Corynebacterium haemolyticum*' (MacLean, Liebow & Rosenberg) in the genus *Arcanobacterium* gen. nov. as *Arcanobacterium haemolyticum* nom.rev. comb. nov. *J Gen Microbiol*. 1982;128:1279–1281.
185. Ramos CP, Foster G, Collins MD. Phylogenetic analysis of the genus *Actinomyces* based on 16S rRNA gene sequences: description of *Arcanobacterium phocae* sp. nov., *Arcanobacterium bernardiae* comb. nov., and *Arcanobacterium pyogenes* comb. nov. *Int J Syst Bacteriol*. 1997;47:46–53.
186. Yassin AF, Hupfer H, Siering C, et al. Comparative chemotaxonomic and phylogenetic studies on the genus *Arcanobacterium* Collins et al. 1982 emend. Lehnen et al. 2006: proposal for *Trueperella* gen. nov. and emended description of the genus *Arcanobacterium*. *Int J Syst Evol Microbiol*. 2011;61(Pt 6):1265–1274.
187. Maclean PD, Liebow AA, Rosenberg AA. A hemolytic *Corynebacterium* resembling *Corynebacterium ovis* and *Corynebacterium pyogenes* in man. *J Infect Dis*. 1946;79:69–90.
188. Cummings LA, Wu WK, Larson AM, et al. Effects of media, atmosphere, and incubation time on colonial morphology of *Arcanobacterium haemolyticum*. *J Clin Microbiol*. 1993;31:3223–3226.
189. Carlson P, Lounatmaa K, Kontiainen S. Biotypes of *Arcanobacterium haemolyticum*. *J Clin Microbiol*. 1994;32:1654–1657.
190. Sammra O, Friis-Moller A, Balbutskaya A, et al. Phenotypic and genotypic characteristics of *Arcanobacterium haemolyticum* isolated from clinical samples in a Danish hospital. *Folia Microbiol (Praha)*. 2014;59:369–374.
191. Ryan WJ. Throat infection and rash associated with an unusual *Corynebacterium*. *Lancet*. 1972;2:1345–1347.
192. Miller RA, Brancato F, Holmes KK. *Corynebacterium hemolyticum* as a cause of pharyngitis and scarlatiniform rash in young adults. *Ann Intern Med*. 1986;105:867–872.
193. Banck G, Nyman M. Tonsillitis and rash associated with *Corynebacterium haemolyticum*. *J Infect Dis*. 1986;154:1037–1040.
194. Carlson P, Renkonen OV, Kontiainen S. *Arcanobacterium haemolyticum* and streptococcal pharyngitis. *Scand J Infect Dis*. 1994;26:283–287.
195. Mackenzie A, Fuite LA, Chan FT, et al. Incidence and pathogenicity of *Arcanobacterium haemolyticum* during a 2-year study in Ottawa. *Clin Infect Dis*. 1995;21:177–181.
196. Miyamoto H, Suzuki T, Murakami S, et al. Bacteriological characteristics of *Arcanobacterium haemolyticum* isolated from seven patients with skin and soft-tissue infections. *J Med Microbiol*. 2015;64(Pt 4):369–374.
197. Lowe CF, Bernard KA, Romney MG. Cutaneous diphtheria in the urban poor population of Vancouver, British Columbia, Canada: a 10-year review. *J Clin Microbiol*. 2011;49:2664–2666.
198. Skov RL, Sanden AK, Danchell VH, et al. Systemic and deep-seated infections caused by *Arcanobacterium haemolyticum*. *Eur J Clin Microbiol Infect Dis*. 1998;17:578–582.
199. Younus F, Chua A, Tortora G, et al. Lemierre's disease caused by co-infection of *Arcanobacterium haemolyticum* and *Fusobacterium necrophorum*: a case report. *J Infect*. 2002;45:114–117.
200. Fernandez-Suarez A, Benitez JM, Vidal AM, et al. Lemierre's syndrome and septicaemia caused solely by *Arcanobacterium haemolyticum* in a young immunocompetent patient. *J Med Microbiol*. 2009;58(Pt 12):1645–1648.
201. Tan TY, Ng SY, Thomas H, et al. *Arcanobacterium haemolyticum* bacteraemia and soft-tissue infections: case report and review of the literature. *J Infect*. 2006;53:e69–e74.
202. Stone LA, Harshbarger RJ 3rd. Orbital necrotizing fasciitis and osteomyelitis caused by *Arcanobacterium haemolyticum*: a case report. *Ophthal Plast Reconstr Surg*. 2015;31:e31–e33.
203. Ramey NA, Burkat CN. *Arcanobacterium hemolyticum* orbital cellulitis: a rare but aggressive disease. *Ophthal Plast Reconstr Surg*. 2013;29:e69–e72.
204. Carlson P, Kontiainen S, Renkonen OV. Antimicrobial susceptibility of *Arcanobacterium haemolyticum*. *Antimicrob Agents Chemother*. 1994;38:142–143.
205. Garcia-de-la-Fuente C, Ruiz De Alegría C, Cano ME, et al. Phenotypic and molecular characterization of *Arcanobacterium haemolyticum* isolated from clinical samples. *Diagn Microbiol Infect Dis*. 2012;72:1–7.
206. Jost BH, Songer JG, Billington SJ. Identification of a second *Arcanobacterium pyogenes* neuraminidase and involvement of neuraminidase activity in host cell adhesion. *Infect Immun*. 2002;70:1106–1112.
207. Gahrn-Hansen B, Frederiksen W. Human infections with *Actinomyces pyogenes (Corynebacterium pyogenes)*. *Diagn Microbiol Infect Dis*. 1992;15:349–354.
208. Kavitha K, Latha R, Udayashankar C, et al. Three cases of *Arcanobacterium pyogenes*-associated soft tissue infection. *J Med Microbiol*. 2010;59(Pt 6):736–739.
209. Plamondon M, Martinez G, Raynal L, et al. A fatal case of *Arcanobacterium pyogenes* endocarditis in a man with no identified animal contact: case report and review of the literature. *Eur J Clin Microbiol Infect Dis*. 2007;26:663–666.
210. Chesdachai S, Larbcharoensub N, Chansoon T, et al. *Arcanobacterium pyogenes* endocarditis: a case report and literature review. *Southeast Asian J Trop Med Public Health*. 2014;45:142–148.
211. Ieven M, Verhoeven J, Gentens P, et al. Severe infection due to *Actinomyces bernardiae*: case report. *Clin Infect Dis*. 1996;22:157–158.
212. Clarke TM, Citron DM, Towfiqh S. The conundrum of the gram-positive rod: are we missing important pathogens in complicated skin and soft-tissue infections? A case report and review of the literature. *Surg Infect (Larchmt)*. 2010;11:65–72.
213. Gilarranz R, Chamizo F, Horcajada I, et al. Prosthetic joint infection caused by *Trueperella bernardiae*. *J Infect Chemother*. 2016;22:642–644.
214. Rattes AL, Araujo MR, Federico MP, et al. *Trueperella bernardiae*: first report of wound infection post laparoscopic surgery. *Clin Case Rep*. 2016;4:812–815.
215. Funke G, Stubbs S, Altwegg M, et al. *Turicella otitidis* gen. nov., sp. nov., a coryneform bacterium isolated from patients with otitis media. *Int J Syst Bacteriol*. 1994;44:270–273.
216. Gomez-Garces JL, Alos JI, Tamayo J. In vitro activity of linezolid and 12 other antimicrobials against coryneform bacteria. *Int J Antimicrob Agents*. 2007;29:688–692.

217. Funke G, Pagano-Niederer M, Sjoden B, et al. Characteristics of *Arthrobacter cumminsii*, the most frequently encountered *Arthrobacter* species in human clinical specimens. *J Clin Microbiol*. 1998;36:1539–1543.
218. Mages IS, Frodl R, Bernard KA, et al. Identities of *Arthrobacter* spp. and *Arthrobacter*-like bacteria encountered in human clinical specimens. *J Clin Microbiol*. 2008;46:2980–2986.
219. Shigeta N, Ozaki K, Hori K, et al. An *Arthrobacter* spp. bacteremia leading to fetal death and maternal disseminated intravascular coagulation. *Fetal Pediatr Pathol*. 2013;31:25–31.
220. Gruner E, Steigerwalt AG, Hollis DG, et al. Human infections caused by *Brevibacterium casei*, formerly CDC groups B-1 and B-3. *J Clin Microbiol*. 1994;32:1511–1518.
221. Funke G, Carlotti A. Differentiation of *Brevibacterium* spp. encountered in clinical specimens. *J Clin Microbiol*. 1994;32:1729–1732.
222. Keusch S, Speich R, Treder U, et al. Central venous catheter infections in outpatients with pulmonary hypertension treated with continuous iloprost. *Respiration*. 2013;86:402–406.
223. Kumar VA, Augustine D, Panikar D, et al. *Brevibacterium casei* as a cause of brain abscess in an immunocompetent patient. *J Clin Microbiol*. 2011;49:4374–4376.
224. Althaf MM, Abdelsalam MS, Alsunaid MS, et al. *Brevibacterium casei* isolated as a cause of relapsing peritonitis. *BMJ Case Rep*. 2014;2014.
225. Troxler R, Funke G, Von Graevenitz A, et al. Natural antibiotic susceptibility of recently established coryneform bacteria. *Eur J Clin Microbiol Infect Dis*. 2001;20:315–323.
226. Wauter G, Haase G, Avesani V, et al. Identification of a novel *Brevibacterium* species isolated from humans and description of *Brevibacterium sanguinis* sp. nov. *J Clin Microbiol*. 2004;42:2829–2832.
227. Manetos CM, Pavlidis AN, Kallistratos MS, et al. Native aortic valve endocarditis caused by *Brevibacterium epidermidis* in an immunocompetent patient. *Am J Med Sci*. 2011;342:257–258.
228. Fe Talento A, Malnick H, Cotter M, et al. *Brevibacterium otitidis*: an elusive cause of neurosurgical infection. *J Med Microbiol*. 2013;62(Pt 3):486–488.
229. Funke G, Stubbs S, Pfyffer GE, et al. Characteristics of CDC group 3 and group 5 coryneform bacteria isolated from clinical specimens and assignment to the genus *Dermabacter*. *J Clin Microbiol*. 1994;32:1223–1228.
230. Gruner E, Steigerwalt AG, Hollis DG, et al. Recognition of *Dermabacter hominis*, formerly CDC fermentative coryneform group 3 and group 5, as a potential human pathogen. *J Clin Microbiol*. 1994;32:1918–1922.
231. Fernandez-Natal I, Saez-Nieto JA, Medina-Pascual MJ, et al. *Dermabacter hominis*: a usually daptomycin-resistant gram-positive organism infrequently isolated from human clinical samples. *New Microbes New Infect*. 2013;1:35–40.
232. Lesher RJ, Gerencser VF, Morrison DJ. Presence of *Rothia dentocariosa* strain 477 serotype 2 in gingiva of patients with inflammatory periodontal disease. *J Dent Res*. 1977;56:189.
233. Boudewijns M, Magerman K, Verhaegen J, et al. *Rothia dentocariosa*, endocarditis and mycotic aneurysms: case report and review of the literature. *Clin Microbiol Infect*. 2003;9:222–229.
234. Fridman D, Chaudhry A, Makaryus J, et al. *Rothia dentocariosa* endocarditis: an especially rare case in a previously healthy man. *Tex Heart Inst J*. 2016;43:255–257.
235. Ramanan P, Barreto JN, Osmon DR, et al. *Rothia* bacteremia: a 10-year experience at Mayo Clinic, Rochester, Minnesota. *J Clin Microbiol*. 2014;52:3184–3189.
236. Abidi MZ, Ledeboer N, Banerjee A, et al. Morbidity and mortality attributable to *Rothia* bacteremia in neutropenic and nonneutropenic patients. *Diagn Microbiol Infect Dis*. 2016;85:116–120.
237. Maraki S, Papadakis IS. *Rothia mucilaginosa* pneumonia: a literature review. *Infect Dis (Lond)*. 2015;47:125–129.
238. Gosmanova EO, Garrett TR, Wall BM. Peritonitis caused by *Rothia mucilaginosa* in a peritoneal dialysis patient. *Am J Med Sci*. 2013;346:517–518.
239. Morgan EA, Henrich TJ, Jarell AD, et al. Infectious granulomatous dermatitis associated with *Rothia mucilaginosa* bacteremia: a case report. *Am J Dermatopathol*. 2010;32:175–179.
240. Rihs JD, McNeil MM, Brown JM, et al. *Oerskovia xanthineolytica* implicated in peritonitis associated with peritoneal dialysis: case report and review of *Oerskovia* infections in humans. *J Clin Microbiol*. 1990;28:1934–1937.
241. Urbina BY, Gohh R, Fischer SA. *Oerskovia xanthineolytica* endocarditis in a renal transplant patient: case report and review of the literature. *Transpl Infect Dis*. 2003;5:195–198.
242. Niamut SM, van der Vorm ER, van Luyn-Wiegers CG, et al. *Oerskovia xanthineolytica* bacteremia in an immunocompromised patient without a foreign body. *Eur J Clin Microbiol Infect Dis*. 2003;22:274–275.
243. Brown JM, Steigerwalt AG, Morey RE, et al. Characterization of clinical isolates previously identified as *Oerskovia turbata*: proposal of *Cellulosimicrobium funkei* sp. nov. and emended description of the genus *Cellulosimicrobium*. *Int J Syst Evol Microbiol*. 2006; 56(Pt 4):801–804.
244. Tucker JD, Montecino R, Winograd JM, et al. Pyogenic flexor tenosynovitis associated with *Cellulosimicrobium cellulans*. *J Clin Microbiol*. 2008;46:4106–4108.
245. Kim JS, Won Lee T, Gyoo Ihm C, et al. CAPD peritonitis caused by co-infection with *Cellulosimicrobium cellulans* (*Oerskovia xanthineolytica*) and *Enterobacter cloacae*: a case report and literature review. *Intern Med*. 2015;54: 627–630.
246. Funke G, Falsen E, Barreau C. Primary identification of *Microbacterium* spp. encountered in clinical specimens as CDC coryneform group A-4 and group A-5 bacteria. *J Clin Microbiol*. 1995;33:188–192.
247. Takeuchi M, Hatano K. Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins et al. in a redefined genus *Microbacterium*. *Int J Syst Bacteriol*. 1998;48(Pt 3):739–747.
248. Funke G, Haase G, Schnitzler N, et al. Endophthalmitis due to *Microbacterium* species: case report and review of *Microbacterium* infections. *Clin Infect Dis*. 1997;24:713–716.
249. Buss SN, Starlin R, Iwen PC. Bacteremia caused by *Microbacterium binotii* in a patient with sickle cell anemia. *J Clin Microbiol*. 2014;52:379–381.
250. Miyamoto M, Sakurada T, Oishi D, et al. The first case report of peritoneal dialysis related peritonitis caused by *Microbacterium paraoxydans*. *Clin Nephrol*. 2013;79:402–406.
251. Gneiding K, Frodl R, Funke G. Identities of *Microbacterium* spp. encountered in human clinical specimens. *J Clin Microbiol*. 2008;46:3646–3652.
252. Evtushenko LI, Dorofeeva LV, Subbotin SA, et al. *Leifsonia poae* gen. nov., sp nov., isolated from nematode galls on *Poa annua*, and reclassification of '*Corynebacterium aquaticum*' Leifson 1962 as *Leifsonia aquatica* (ex Leifson 1962) gen. nov., nom. rev., comb. nov and *Clavibacter xyli* Davis et al. 1984 with two subspecies as *Leifsonia xyli* (Davis et al. 1984) gen. nov., comb. nov. *Int J Syst Evol Microbiol*. 2000;50:371–380.
253. Porte L, Soto A, Andrighetti D, et al. Catheter-associated bloodstream infection caused by *Leifsonia aquatica* in a haemodialysis patient: a case report. *J Med Microbiol*. 2012;61(Pt 6):868–873.
254. Han L, Lei JE, Wang X, et al. Septicemia caused by *Leifsonia aquatica* in a healthy patient after retinal reattachment surgery. *J Clin Microbiol*. 2013;51: 3886–3888.
255. Goodfellow M, Sangal V, Jones AL, et al. Charting stormy waters: a commentary on the nomenclature of the equine pathogen variously named *Prescottella equi*, *Rhodococcus equi* and *Rhodococcus hoagii*. *Equine Vet J*. 2015;47: 508–509.
256. Walsh RD, Schoch PE, Cunha BA. Rhodococcus. *Infect Control Hosp Epidemiol*. 1993;14:282–287.
257. Yamshchikov AV, Schuetz A, Lyon GM. *Rhodococcus equi* infection. *Lancet Infect Dis*. 2010;10:350–359.
258. Topino S, Galati V, Grilli E, et al. *Rhodococcus equi* infection in HIV-infected individuals: case reports and review of the literature. *AIDS Patient Care STDS*. 2010;24:211–222.
259. Weinstock DM, Brown AE. *Rhodococcus equi*: an emerging pathogen. *Clin Infect Dis*. 2002;34:1379–1385.
260. Verville TD, Huycke MM, Greenfield RA, et al. *Rhodococcus equi* infections of humans. 12 cases and a review of the literature. *Medicine (Baltimore)*. 1994;73:119–132.
261. Takai S, Ohbushi S, Koike K, et al. Prevalence of virulent *Rhodococcus equi* in isolates from soil and feces of horses from horse breeding farms with and without endemic infections. *J Clin Microbiol*. 1991;29:2887–2889.
262. Kedlaya I, Ing MB, Wong SS. *Rhodococcus equi* infections in immunocompetent hosts: case report and review. *Clin Infect Dis*. 2001;32:E39–E46.
263. Herath S, Lewis C, Nisbet M. Increasing awareness of *Rhodococcus equi* pulmonary infection in the immunocompetent adult: a rare infection with poor prognosis. *N Z Med J*. 2013;126:165–174.
264. Scotton PG, Tonon E, Giobbia M, et al. *Rhodococcus equi* nosocomial meningitis cured by levofloxacin and shunt removal. *Clin Infect Dis*. 2000;30:223–224.
265. Arlotti M, Zoboli G, Moscatelli GL, et al. *Rhodococcus equi* infection in HIV-positive subjects: a retrospective analysis of 24 cases. *Scand J Infect Dis*. 1996;28:463–467.
266. Rasmussen TT, Kirkeby LP, Poulsen K, et al. Resident aerobic microbiota of the adult human nasal cavity. *APMIS*. 2000;108:663–675.
267. Nordmann P, Nicolas MH, Gutmann L. Penicillin-binding proteins of *Rhodococcus equi*: potential role in resistance to imipenem. *Antimicrob Agents Chemother*. 1993;37:1406–1409.
268. Wang T, Kong F, Chen S, et al. Improved identification of *Gordonia*, *Rhodococcus* and *Tsukamurella* species by 5'-end 16S rRNA gene sequencing. *Pathology*. 2011;43:58–63.
269. Bharadwaj R, Swaminathan S, Salimnia H, et al. Clinical impact of the use of 16S rRNA sequencing method for the identification of "difficult-to-identify" bacteria in immunocompromised hosts. *Transpl Infect Dis*. 2012;14:206–212.
270. Hsueh PR, Lee TF, Du SH, et al. Bruker biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of *Nocardia*, *Rhodococcus*, *Kocuria*, *Gordonia*, *Tsukamurella*, and *Listeria* species. *J Clin Microbiol*. 2014;52:2371–2379.
271. Miranda-Caso Luengo AA, Miranda-CasoLuengo R, Lieggi NT, et al. A real-time impedance based method to assess *Rhodococcus equi* virulence. *PLoS ONE*. 2013;8:e60612.
272. Drancourt M, Bonnet E, Gallais H, et al. *Rhodococcus equi* infection in patients with AIDS. *J Infect*. 1992;24:123–131.
273. Ramos-Vivas J, Pilares-Ortega L, Remuzgo-Martinez S, et al. *Rhodococcus equi* human clinical isolates enter and survive within human alveolar epithelial cells. *Microbes Infect*. 2011;13:438–446.
274. Giguere S, Hondalus MK, Yager JA, et al. Role of the 85-kilobase plasmid and plasmid-encoded virulence-associated protein A in intracellular survival and virulence of *Rhodococcus equi*. *Infect Immun*. 1999;67:3548–3557.
275. Oldfield C, Bonella H, Renwick L, et al. Rapid determination of vapA/vapB genotype in *Rhodococcus equi* using a differential polymerase chain reaction method. *Antonie Van Leeuwenhoek*. 2004;85:317–326.
276. Fidvi SA, Brudnicki AR, Chowdhury MI, et al. Cavitary *Rhodococcus equi* pneumonia with endobronchial granulomas: report of an unusual case. *Pediatr Radiol*. 2003;33:140–142.
277. Guerrero MF, Ramos JM, Renedo G, et al. Pulmonary malacoplakia associated with *Rhodococcus equi* infection in patients with AIDS: case report and review. *Clin Infect Dis*. 1999;28:1334–1336.
278. Harvey RL, Sunstrum JC. *Rhodococcus equi* infection in patients with and without human immunodeficiency virus infection. *Rev Infect Dis*. 1991;13:139–145.
279. Lasky JA, Pulkingham N, Powers MA, et al. *Rhodococcus equi* causing human pulmonary infection: review of 29 cases. *South Med J*. 1991;84:1217–1220.
280. Donisi A, Suardi MG, Casari S, et al. *Rhodococcus equi* infection in HIV-infected patients. *AIDS*. 1996;10:359–362.
281. Hsueh PR, Hung CC, Teng LJ, et al. Report of invasive *Rhodococcus equi* infections in Taiwan, with an emphasis on the emergence of multidrug-resistant strains. *Clin Infect Dis*. 1998;27:370–375.
282. Munoz P, Burillo A, Palomo J, et al. *Rhodococcus equi* infection in transplant recipients: case report and review of the literature. *Transplantation*. 1998;65:449–453.
283. Linder R. *Rhodococcus equi* and *Arcanobacterium haemolyticum*: two "coryneform" bacteria increasingly recognized as agents of human infection. *Emerg Infect Dis*. 1997;3:145–153.
284. Farina C, Ferruzzi S, Mamprin F, et al. *Rhodococcus equi* infection in non-HIV-infected patients. Two case reports and review. *Clin Microbiol Infect*. 1997;3:12–18.
285. Akan H, Akova M, Ataoglu H, et al. *Rhodococcus equi* and *Nocardia brasiliensis* infection of the brain and liver in a patient with acute nonlymphoblastic leukemia. *Eur J Clin Microbiol Infect Dis*. 1998;17:737–739.
286. Sigler E, Miskin A, Shtlarid M, et al. Fever of unknown origin and anemia with *Rhodococcus equi* infection in an immunocompetent patient. *Am J Med*. 1998;104:510.
287. Linares MJ, Lopez-Encuentra A, Perea S. Chronic pneumonia caused by *Rhodococcus equi* in a patient without impaired immunity. *Eur Respir J*. 1997;10:248–250.
288. Munoz P, Palomo J, Guembe P, et al. Lung nodular lesions in heart transplant recipients. *J Heart Lung Transplant*. 2000;19:660–667.
289. Torres-Tortosa M, Arrizabalaga J, Villanueva JL, et al. Prognosis and clinical evaluation of infection caused by *Rhodococcus equi* in HIV-infected patients: a multicenter study of 67 cases. *Chest*. 2003;123:1970–1976.
290. Antinori S, Esposito R, Cernuschi M, et al. Disseminated *Rhodococcus equi* infection initially presenting as foot mycetoma in an HIV positive patient. *AIDS*. 1992;6:740–742.
291. Matsushita H, Hanayama N, Hobo K, et al. Infectious endocarditis caused by *Rhodococcus equi*. *Ann Thorac Surg*. 2010;89:957–959.

292. Chow KM, Szeto CC, Chow VC, et al. *Rhodococcus equi* peritonitis in continuous ambulatory peritoneal dialysis. *J Nephrol*. 2003;16:736–739.
293. Strunk T, Gardiner K, Simmer K, et al. *Rhodococcus equi* meningitis after ventriculoperitoneal shunt insertion in a preterm infant. *Pediatr Infect Dis J*. 2007;26:1076–1077.
294. Al Akhrass F, Al Wohoush I, Chaftari AM, et al. *Rhodococcus* bacteremia in cancer patients is mostly catheter related and associated with biofilm formation. *PLoS ONE*. 2012;7:e32945.
295. Cornish N, Washington JA. *Rhodococcus equi* infections: clinical features and laboratory diagnosis. *Curr Clin Top Infect Dis*. 1999;19:198–215.
296. Tuon FF, Siciliano RF, Al-Musawi T, et al. *Rhodococcus equi* bacteremia with lung abscess misdiagnosed as *Corynebacterium*. A report of 2 cases. *Clinics*. 2007;62:795–798.
297. Rodriguez Arrondo F, von Wichmann MA, Arrizabalaga J, et al. [Pulmonary cavitation lesions in patients infected with the human immunodeficiency virus: an analysis of a series of 78 cases]. *Med Clin (Barc)*. 1998;111:725–730.
298. Savini V, Fazii P, Favaro M, et al. Tuberculosis-like pneumonias by the aerobic actinomycetes *Rhodococcus*, *Tsukamurella* and *Gordonia*. *Microbes Infect*. 2012;14:401–410.
299. Marchiori E, Muller NL, de Mendonca RG, et al. *Rhodococcus equi* pneumonia in AIDS: high-resolution CT findings in five patients. *Br J Radiol*. 2005;78:783–786.
300. Kamboj M, Kalra A, Kak V. *Rhodococcus equi* brain abscess in a patient without HIV. *J Clin Pathol*. 2005;58:423–425.
301. Ulivieri S, Oliveri G. Cerebellar abscess due to *Rhodococcus equi* in an immunocompetent patient: case report and literature review. *J Neurosurg Sci*. 2006;50:127–129.
302. Rallis G, Dais P, Gkinis G, et al. Acute osteomyelitis of the mandible caused by *Rhodococcus equi* in an immunocompromised patient: a case report and literature review. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2012;114:e1–e5.
303. Meeuse JJ, Sprenger HG, van Assen S, et al. *Rhodococcus equi* infection after alemtuzumab therapy for T-cell prolymphocytic leukemia. *Emerg Infect Dis*. 2007;13:1942–1943.
304. Shahani L. *Rhodococcus equi* pneumonia and sepsis in an allogeneic haematopoietic stem cell transplant recipient. *BMJ Case Rep*. 2014;2014.
305. Darraj M, Fainstein R, Kasper K, et al. Immune reconstitution syndrome secondary to *Rhodococcus equi* infection in a patient with HIV and Burkitt's lymphoma. *J Infect Public Health*. 2017;10:224–227.
306. Munoz P, Palomo J, Guinea J, et al. Relapsing *Rhodococcus equi* infection in a heart transplant recipient successfully treated with long-term linezolid. *Diagn Microbiol Infect Dis*. 2008;60:197–199.
307. Menon V, Gottlieb T, Gallagher M, et al. Persistent *Rhodococcus equi* infection in a renal transplant patient: case report and review of the literature. *Transpl Infect Dis*. 2012;14:E126–E133.
308. Speck D, Koneth I, Diethelm M, et al. A pulmonary mass caused by *Rhodococcus equi* infection in a renal transplant recipient. *Nat Clin Pract Nephrol*. 2008;4:398–403.
309. Cronin SM, Abidi MH, Shearer CJ, et al. *Rhodococcus equi* lung infection in an allogeneic hematopoietic stem cell transplant recipient. *Transpl Infect Dis*. 2008;10:48–51.
310. Gabriels P, Joosen H, Put E, et al. Recurrent *Rhodococcus equi* infection with fatal outcome in an immunocompetent patient. *Eur J Clin Microbiol Infect Dis*. 2006;25:46–48.
311. Watanabe H, Asoh N, Kobayashi S, et al. Clinical and microbiological characteristics of community-acquired pneumonia among human immunodeficiency virus-infected patients in northern Thailand. *J Infect Chemother*. 2008;14:105–109.
312. Spiliopoulou A, Assimakopoulos SF, Foka A, et al. Pulmonary infection by *Rhodococcus equi* presenting with positive Ziehl-Neelsen stain in a patient with human immunodeficiency virus: a case report. *J Med Case Rep*. 2014;8:423.
313. Gundelly P, Suzuki Y, Ribes JA, et al. Differences in *Rhodococcus equi* infections based on immune status and antibiotic susceptibility of clinical isolates in a case series of 12 patients and cases in the literature. *Biomed Res Int*. 2016;2016:2737295.
314. Bowersock TL, Salmon SA, Portis ES, et al. MICs of oxazolidinones for *Rhodococcus equi* strains isolated from humans and animals. *Antimicrob Agents Chemother*. 2000;44:1367–1369.
315. Jacks SS, Giguere S, Nguyen A. In vitro susceptibilities of *Rhodococcus equi* and other common equine pathogens to azithromycin, clarithromycin, and 20 other antimicrobials. *Antimicrob Agents Chemother*. 2003;47:1742–1745.
316. Puthucheary SD, Sangkar V, Hafeez A, et al. *Rhodococcus equi*—an emerging human pathogen in immunocompromised hosts: a report of four cases from Malaysia. *Southeast Asian J Trop Med Public Health*. 2006;37:157–161.
317. Rolston KV, Frisbee-Hume S, LeBlanc B, et al. In vitro antimicrobial activity of moxifloxacin compared to other quinolones against recent clinical bacterial isolates from hospitalized and community-based cancer patients. *Diagn Microbiol Infect Dis*. 2003;47:441–449.
318. Rolston KV, Vaziri I, Frisbee-Hume S, et al. In vitro antimicrobial activity of gatifloxacin compared with other quinolones against clinical isolates from cancer patients. *Chemotherapy*. 2004;50:214–220.
319. Nordmann P, Kerestedjian JJ, Ronco E. Therapy of *Rhodococcus equi* disseminated infections in nude mice. *Antimicrob Agents Chemother*. 1992;36:1244–1248.
320. Giguere S, Lee EA, Guldbech KM, et al. In vitro synergy, pharmacodynamics, and postantibiotic effect of 11 antimicrobial agents against *Rhodococcus equi*. *Vet Microbiol*. 2012;160:207–213.
321. Asoh N, Watanabe H, Fines-Guyon M, et al. Emergence of rifampin-resistant *Rhodococcus equi* with several types of mutations in the rpoB gene among AIDS patients in northern Thailand. *J Clin Microbiol*. 2003;41:2337–2340.
322. Giguere S, Berghaus LJ, Willingham-Lane JM. Antimicrobial resistance in *Rhodococcus equi*. *Microbiol Spectr*. 2017;5.
323. Niwa H, Lasker BA. Mutant selection window and characterization of allelic diversity for ciprofloxacin-resistant mutants of *Rhodococcus equi*. *Antimicrob Agents Chemother*. 2010;54:3520–3523.
324. Tse KC, Tang SC, Chan TM, et al. *Rhodococcus* lung abscess complicating kidney transplantation: successful management by combination antibiotic therapy. *Transpl Infect Dis*. 2008;10:44–47.
325. Napoleao F, Damasco PV, Camello TCF, et al. Pyogenic liver abscess due to *Rhodococcus equi* in an immunocompetent host. *J Clin Microbiol*. 2005;43:1002–1004.
326. Gundelly P, Thornton A, Greenberg RN, et al. *Rhodococcus equi* pericarditis in a patient living with HIV/AIDS. *J Int Assoc Provid AIDS Care*. 2014;13:309–312.
327. Ursales A, Klein JA, Beal SG, et al. Antibiotic failure in a renal transplant patient with *Rhodococcus equi* infection: an indication for surgical lobectomy. *Transpl Infect Dis*. 2014;16:1019–1023.
328. Buchman AL, McNeil MM, Brown JM, et al. Central venous catheter sepsis caused by unusual *Gordona* (*Rhodococcus*) species: identification with a digoxigenin-labeled rDNA probe. *Clin Infect Dis*. 1992;15:694–697.
329. Drancourt M, McNeil MM, Brown JM, et al. Brain abscess due to *Gordona terrae* in an immunocompromised child: case report and review of infections caused by *G. terrae*. *Clin Infect Dis*. 1994;19:258–262.
330. Drancourt M, Pelletier J, Cherif AA, et al. *Gordona terrae* central nervous system infection in an immunocompetent patient. *J Clin Microbiol*. 1997;35:379–382.
331. Lesens O, Hansmann Y, Riegel P, et al. Bacteremia and endocarditis caused by a *Gordonia* species in a patient with a central venous catheter. *Emerg Infect Dis*. 2000;6:382–385.
332. Pham AS, De I, Rolston KV, et al. Catheter-related bacteremia caused by the nocardioform actinomycete *Gordonia terrae*. *Clin Infect Dis*. 2003;36:524–527.
333. Blaschke AJ, Bender J, Byington CL, et al. *Gordonia* species: emerging pathogens in pediatric patients that are identified by 16S ribosomal RNA gene sequencing. *Clin Infect Dis*. 2007;45:483–486.
334. Moser BD, Pellegrini GJ, Lasker BA, et al. Pattern of antimicrobial susceptibility obtained from blood isolates of a rare but emerging human pathogen, *Gordonia polyisoprenivorans*. *Antimicrob Agents Chemother*. 2012;56:4991–4993.
335. Renvoise A, Harle JR, Raoult D, et al. *Gordonia sputi* bacteremia. *Emerg Infect Dis*. 2009;15:1535–1537.
336. Hou C, Yang Y, Li Z. A Chinese patient with peritoneal dialysis-related peritonitis caused by *Gordonia terrae*: a case report. *BMC Infect Dis*. 2017;17:179.
337. Andalibi F, Fatahi-Bafghi M. *Gordonia*: isolation and identification in clinical samples and role in biotechnology. *Folia Microbiol (Praha)*. 2017;62:245–252.
338. Drzyzga O. The strengths and weaknesses of *Gordonia*: a review of an emerging genus with increasing biotechnological potential. *Crit Rev Microbiol*. 2012;38:300–316.
339. Richet HM, Craven PC, Brown JM, et al. A cluster of *Rhodococcus* (*Gordona*) *bronchialis* sternal-wound infections after coronary-artery bypass surgery. *N Engl J Med*. 1991;324:104–109.
340. Sng LH, Koh TH, Toney SR, et al. Bacteremia caused by *Gordonia bronchialis* in a patient with sequestrated lung. *J Clin Microbiol*. 2004;42:2870–2871.
341. Werno AM, Anderson TP, Chambers ST, et al. Recurrent breast abscess caused by *Gordonia bronchialis* in an immunocompetent patient. *J Clin Microbiol*. 2005;43:3009–3010.
342. Rodriguez-Lozano J, Perez-Llantada E, Aguero J, et al. Sternal wound infection caused by *Gordonia bronchialis*: identification by MALDI-TOF MS. *JMM Case Rep*. 2016;3:e005067.
343. Siddiqui N, Toumeh A, Georgescu C. Tibial osteomyelitis caused by *Gordonia bronchialis* in an immunocompetent patient. *J Clin Microbiol*. 2012;50:3119–3121.
344. Johnson JA, Onderdonk AB, Cosimi LA, et al. *Gordonia bronchialis* bacteremia and pleural infection: case report and review of the literature. *J Clin Microbiol*. 2011;49:1662–1666.
345. Wright SN, Gerry JS, Busowski MT, et al. *Gordonia bronchialis* sternal wound infection in 3 patients following open heart surgery: intraoperative transmission from a healthcare worker. *Infect Control Hosp Epidemiol*. 2012;33:1238–1241.
346. Osoagbaka OU. Evidence for the pathogenic role of *Rhodococcus* species in pulmonary diseases. *J Appl Bacteriol*. 1989;66:497–506.
347. Shapiro CL, Haft RF, Gantz NM, et al. *Tsukamurella paurometabolum*: a novel pathogen causing catheter-related bacteremia in patients with cancer. *Clin Infect Dis*. 1992;14:200–203.
348. Almehmi A, Pfister AK, McCowan R, et al. Implantable cardioverter-defibrillator infection caused by *Tsukamurella*. *W V Med J*. 2004;100:185–186.
349. Woo PC, Ngan AH, Lau SK, et al. *Tsukamurella* conjunctivitis: a novel clinical syndrome. *J Clin Microbiol*. 2003;41:3368–3371.
350. Elshibly S, Doherty J, Xu J, et al. Central line-related bacteraemia due to *Tsukamurella tyrosinosolvens* in a haematology patient. *Ulster Med J*. 2005;74:43–46.
351. Alcaide ML, Espinoza L, Abbo L. Cavitary pneumonia secondary to *Tsukamurella* in an AIDS patient. First case and a review of the literature. *J Infect*. 2004;49:17–19.
352. Tsukamura M, Hikosaka K, Nishimura K, et al. Severe progressive subcutaneous abscesses and necrotizing tenosynovitis caused by *Rhodococcus aurantiacus*. *J Clin Microbiol*. 1988;26:201–205.
353. Prinz G, Ban E, Fekete S, et al. Meningitis caused by *Gordona aurantiaca (Rhodococcus aurantiacus)*. *J Clin Microbiol*. 1985;22:472–474.
354. Bouza E, Perez-Parra A, Rosal M, et al. *Tsukamurella*: a cause of catheter-related bloodstream infections. *Eur J Clin Microbiol Infect Dis*. 2009;28:203–210.
355. Liu CY, Lai CC, Lee MR, et al. Clinical characteristics of infections caused by *Tsukamurella* spp. and antimicrobial susceptibilities of the isolates. *Int J Antimicrob Agents*. 2011;38:534–537.
356. Yang L, Cao Y, Dan Z, et al. Community-acquired *Tsukamurella* pneumonia in a young immunocompetent adult: a case misdiagnosed as pulmonary tuberculosis and literature review. *Postgrad Med*. 2017;129:563–566.
357. Stanley T, Crothers L, McCalmont M, et al. The potential misidentification of *Tsukamurella pulmonis* as an atypical *Mycobacterium* species: a cautionary tale. *J Med Microbiol*. 2006;55:475–476.
358. von Below H, Wilk CM, Schaal KP, et al. *Rhodococcus luteus* and *Rhodococcus erythropolis* chronic endophthalmitis after lens implantation. *Am J Ophthalmol*. 1991;112:596–597.
359. Brown E, Hendler E. *Rhodococcus* peritonitis in a patient treated with peritoneal dialysis. *Am J Kidney Dis*. 1989;14:417–418.
360. Vernazza PL, Bodmer T, Galeazzi RL. [*Rhodococcus erythropolis* infection in HIV-associated immunodeficiency]. *Schweiz Med Wochenschr*. 1991;121:1095–1098.
361. Baba H, Nada T, Ohkusu K, et al. First case of bloodstream infection caused by *Rhodococcus erythropolis*. *J Clin Microbiol*. 2009;47:2667–2669.
362. Haburchak DR, Jeffery B, Higbee JW, et al. Infections caused by *Rhodochrous*. *Am J Med*. 1978;65:298–302.
363. Gopaul D, Ellis C, Maki A Jr, et al. Isolation of *Rhodococcus rhodochrous* from a chronic corneal ulcer. *Diagn Microbiol Infect Dis*. 1988;10:185–190.
364. Ramanan P, Deziel PJ, Razonable RR. *Rhodococcus globerulus* bacteremia in an allogeneic hematopoietic stem cell transplant recipient: report of the first transplant case and review of the literature. *Transpl Infect Dis*. 2014;16:484–489.
365. Cuello OH, Caorlin MJ, Reviglio VE, et al. *Rhodococcus globerulus* keratitis after laser in situ keratomileusis. *J Cataract Refract Surg*. 2002;28:2235–2237.
366. DeMarais PL, Kocka FE. *Rhodococcus* meningitis in an immunocompetent host. *Clin Infect Dis*. 1995;20:167–169.

206 Listeria monocytogenes

Jennie E. Johnson and Eleftherios Mylonakis

SHORT VIEW SUMMARY

Diagnosis
- Culture of blood, cerebrospinal fluid, or other normally sterile body fluid or, in the case of gastroenteritis, from stool.
- Serology not useful for invasive disease.

Microbiology
- Short, gram-positive rod; grows readily on blood agar; tumbling motility.
- May be mistaken for diphtheroid contaminant.
- Will grow in refrigerated food.

Epidemiology
- Zoonosis, particularly herd animals.
- Human transmission from contaminated food or from pregnant woman to fetus or newborn.
- Highest food risks from delicatessen-style meats and unpasteurized cheeses.
- Most cases in neonates, pregnant women, adults 60 years old or older, and individuals with impaired cell-mediated immunity resulting from underlying condition (hematologic malignancy, organ or bone marrow transplantation, acquired immunodeficiency syndrome) or therapy (corticosteroids, anti–tumor necrosis factor agents). Notify laboratory for special stool cultures if outbreak of febrile gastroenteritis.

Clinical Settings
- Neonatal sepsis or meningitis.
- Meningitis or focal central nervous system (CNS) lesions in immunosuppressed patients or adults older than 50 years.
- Rhombencephalitis occurs in previously healthy patients who develop fever; cranial nerve palsies; cerebellar signs; and hemiparesis, hemisensory deficits, or both.
- Fever in pregnancy, especially third trimester.
- Outbreak of foodborne febrile gastroenteritis.

Treatment
- Ampicillin (2 g IV every 4 hours); consider adding gentamicin (5 mg/kg/day) for CNS infection or endocarditis.
- Trimethoprim-sulfamethoxazole (5/25 mg/kg IV every 8 hours) for penicillin-allergic patients.

Prevention
- *Pneumocystis* prophylaxis with trimethoprim-sulfamethoxazole likely prevents listeriosis.

DEFINITION

Listeria monocytogenes is a gram-positive bacillus and zoonotic and foodborne pathogen found worldwide that causes listeriosis. Listeriosis spans the clinical spectrum from self-limited febrile gastroenteritis in immunocompetent people to more severe and invasive disease that mostly affects pregnant women, newborn infants, older adults, and individuals with cell-mediated immunodeficiencies. It is a rare but important pathogen because of the populations it infects and its high rates of mortality.

EPIDEMIOLOGY

L. monocytogenes was first described in 1926 after it was identified as the causative pathogen responsible for an outbreak and sudden death of a number of laboratory animals at Cambridge University. Initially named *Bacterium monocytogenes,* it was isolated from infected laboratory animals, a rabbit and a guinea pig, that were also noted to have a peripheral monocytosis.[1] Already known to be an important zoonotic disease infecting more than 40 mammalian and avian species, *L. monocytogenes* was first recognized as a human pathogen in 1929.[2] Additionally, *L. monocytogenes* was known to be transmissible by direct inoculation from an infected animal to a human causing cutaneous infection as well as vertically from mother to fetus causing abortion, stillbirth, or neonatal infection. However, the route of transmission causing bacteremia and meningoencephalitis eluded scientists until 1983, when Schlech and colleagues[3] traced an outbreak of *L. monocytogenes* serotype 4b in Nova Scotia, Canada, to contaminated coleslaw and cabbage. *L. monocytogenes* is now widely recognized as a serious but rare cause of foodborne infections and has the highest case-fatality rate of any foodborne pathogen in the Western Hemisphere.[4–6]

Although most cases of listeriosis are sporadic, *L. monocytogenes* is a cause of major foodborne outbreaks globally.[7,8] Longer incubation periods for listeriosis can affect recall and can make trace-back investigations and identification of the causative foods difficult.[9] Foods that have been linked to outbreaks are those that are at risk for contamination; support the growth of *L. monocytogenes;* may be stored for long periods of time allowing for a higher inoculum of bacteria on ingestion; and are refrigerated, as it can replicate at lower temperatures.[10] *L. monocytogenes* can also withstand high-salt and lower pH environments.[9] Specific foods implicated in outbreaks include soft cheeses; cheeses made from raw milk; raw produce including packaged salads; cantaloupe and sprouts; caramel apples; frozen vegetables; smoked seafood; ice cream; and ready-to-eat meats such as delicatessen meats, hot dogs, and pâté.[11–13] Food contamination can occur at any level of production, from preharvest to food processing. *L. monocytogenes* is a ubiquitous organism found worldwide in water, soil, animal feces, and vegetation. It forms biofilms and can persist in food-processing environments for years.[14] Pasteurization and antimicrobial agents used in foods before and after packaging effectively neutralize *Listeria.* Careful cleaning of food-processing equipment and clean postprocessing handling practices are ways of reducing contamination.[10]

In 2010 listeriosis caused an estimated 23,150 illnesses, 54,463 deaths, and 172,823 disability-adjusted life-years worldwide.[7] Much higher rates in industrialized countries are likely due to more standardized reporting, although other potential factors such as dietary habits, food processing, testing, and host factors may contribute.[15] Seasonal trends have been observed in Europe and the United States with peak incidence of invasive listeriosis cases in the summer months.[16–18]

MICROBIOLOGY

The genus *Listeria* contains 17 species of small, gram-positive, rod-shaped bacteria, of which three, *L. monocytogenes, Listeria ivanovii,* and *Listeria grayi,* are opportunistic pathogens in humans.[19–22] *L. monocytogenes* was the first *Listeria* spp. identified and is responsible for nearly all cases of listeriosis in humans. *L. monocytogenes* is a nonsporulating, catalase-positive, oxidase-negative, flagellated facultative anaerobe that grows optimally at 30°C to 37°C.[23] It demonstrates characteristic tumbling motility at 20°C to 28°C, is able to grow at temperatures as low as 4°C, and is tolerant of high-salt environments.[23,24] Morphologically, *L. monocytogenes* can be decolorized by alcohol during the Gram staining process and appear as gram-variable or gram-negative.[25] Different growth

media may produce short rods, longer rods, or elliptical cocci.[25] For these reasons, it can be mistaken for diphtheroids, *Enterococcus* spp., *Streptococcus pneumoniae, Haemophilus influenzae,* or enteric bacteria on Gram stain.[25] Some commercially available polymerase chain reaction (PCR) film arrays for cerebrospinal fluid (CSF) include *L. monocytogenes* and should facilitate rapid diagnosis.

L. monocytogenes grows well on most routine culture media and usually forms a narrow zone of β-hemolysis on blood agar, although nonhemolytic strains have been described.[19,23] Selective media used to prevent overgrowth from other bacteria present can be used to culture *L. monocytogenes* from nonsterile sources such as the vaginal canal, stool, and food.[26,27]

There are more than 14 serotypes, which are differentiated based on their somatic (O) and flagellar (H) antigens.[28] Serotypes 1/2a, 1/2b, and 4b are implicated in more than 95% of cases of human listeriosis.[11] Various PCR methods can be used to quickly identify the different high-risk serotypes.[29] Whole-genome sequencing has been a valuable tool in outbreak tracing.

PATHOGENESIS

L. monocytogenes is a hardy organism that is adapted to live as a saprophyte in external environments or as an intracellular pathogen in a number of animal hosts.[30] Within its animal hosts, it maintains an arsenal of defensive mechanisms to survive in, respond to, and proliferate in the diverse environments it encounters.[31] *L. monocytogenes* can employ a number of virulence factors such as Internalin A (InlA) encoded by *inlA* and listeriolysin O (LLO) encoded by *hly* to help facilitate entry into and movement through the host cells, to escape phagosomes, and to circumvent the host immune response.[32] Virulence genes are often used as targets for PCR detection and subtyping.[33]

In nearly all cases of infection, *L. monocytogenes* enters into human hosts via the gastrointestinal tract after ingestion of contaminated food. In the stomach, gastric pH of less than 3 is informally bactericidal; however bacterial survival increases at a pH of 3.5 and even more at a pH of 4.[34] This is perhaps why the widespread use of proton pump inhibitors and thus elevated gastric pH has been shown to increase the risk of nonperinatal invasive listeriosis after adjusting for confounding factors.[35] *L. monocytogenes* then moves into the duodenum where the high concentration of bile creates a hostile environment for most microbes.[36] The expression of bile salt hydrolases and the ability to tolerate high-salt conditions allow *L. monocytogenes* not only to survive transit through the duodenum but also to colonize the gallbladder.[37,38] Luminal antibodies have not been shown to be protective against *L. monocytogenes,* whereas innate immune mechanisms such as bactericidal peptides produced by Paneth and epithelial cells are successful host defenses in the small intestine.[37]

The intestinal microbiota also provides an independent and important first line of defense against *L. monocytogenes* infection and indirectly augments host defenses.[37,39] Potential mechanisms of protective properties of commensal bacteria are nutrient competition, contact-dependent inhibition and production of bacterial soluble mediators, and bacteriocins, which are toxic molecules created by certain common gut bacteria.[37] Conversely, dysbiosis, from exposure to antibacterial agents or other causes, can allow for the expansion of *L. monocytogenes* within the intestine. Listeriolysin S is a virulence factor found in epidemic strains of *L. monocytogenes* and the only known bacteriocin produced in the *Listeria* genus that disrupts the host gut microbiota creating a beneficial environment for *L. monocytogenes* during infection.[40]

Once at the intestinal wall, *L. monocytogenes* is able to invade both phagocytic and nonphagocytic cells by employing a number of different strategies. Bacterial virulence factors and surface proteins InlA and InlB bind to the surface receptors E-cadherin and Met on nonphagocytic host cells such as epithelial cells and facilitate entry by receptor-mediated endocytosis.[41] After internalization, the low pH of the phagosome activates LLO, which, along with phospholipases PlcA and PlcB, causes pore formation of the organelle wall and inhibits lysosome fusion, allowing the bacterium to escape.[32,42] Within the cytosol, *L. monocytogenes* is able to replicate with a doubling time of about 1 hour.[42] Bacterial surface protein ActA recruits and binds actin from the host cell to form an actin comet tail at one pole enabling the bacterium to propel itself through the cytoplasm.[43] The bacterium then finds the plasma membrane of the infected host cell. It becomes enveloped in the membrane, extruded from the infected cell, and then phagocytosed by the neighboring cell.[43] It again uses LLO to escape the phagosome, infecting the new host cell and thereby enabling cell-to-cell spread while avoiding the humoral immune system (Fig. 206.1A).[43]

L. monocytogenes is also able to invade goblet cells expressing E-cadherin by InlA-mediated transcytosis and is rapidly transported through the cell, independent of InlB, LLO, and ActA.[44] In some macrophages, *L. monocytogenes* is able to replicate within vacuoles called spacious *Listeria*-containing phagosomes before phagosome escape (Fig. 206.1B).[41]

Having traversed the gastrointestinal border, *L. monocytogenes* then disseminates from lymph nodes into the bloodstream to the spleen and liver and across the blood-brain and placental barriers in the appropriate hosts.[41] Invasion into the central nervous system (CNS) is accomplished by a number of different mechanisms including Internalin receptor-mediated endocytosis through endothelial cells; the neural route, where *L. monocytogenes* migrates from peripheral neurons to central neurons by cell-to-cell spread; and transport inside infected monocytes, the so-called Trojan horse.[45] Transplacental infection likely occurs predominantly by Internalin receptor-mediated endocytosis and cell-to-cell spread but may also occur via inflammation-mediated and primary hematogenous spread.[46,47] Newly identified virulence factors may better explain organ-specific tropism in invasive listeriosis such as InlP and placental tissue.[48]

Despite the ubiquitous and robust nature of *L. monocytogenes,* actual infection is relatively rare owing to active host defenses. Innate and adaptive host defenses against *L. monocytogenes* have been extensively studied in in vivo murine models and in vitro human cells, so much is extrapolated as to how these systems work in humans.[49] The innate immune response is rapid and crucial for host survival.[50] On escape from the phagosome within an infected macrophage, LLO activates at least three separate pathways that produce distinct host responses.[51] One pathway promotes pyroptotic host cell death.[51] Microbial products released from infected macrophages recruit macrophages and promote their differentiation into dendritic cells.[50] The dendritic cells produce inducible nitric oxide synthase and tumor necrosis factor (TNF) to induce microbial killing.[50] Natural killer cells produce interferon-γ that, along with TNF, further activate macrophages, which are, along with neutrophils, directly listeriacidal.[50,52]

Cell-mediated immunity, as opposed to humoral immunity, plays the predominant role in the adaptive response and is necessary for clearance of infection.[53] The innate immune response to *L. monocytogenes* infection is essential for the development of the host adaptive response.[51]

CLINICAL MANIFESTATIONS

As noted earlier, *L. monocytogenes* can cause a broad spectrum of disease ranging from self-limiting febrile gastroenteritis to invasive disease such as bacteremia and meningoencephalitis. Development of invasive disease usually depends on host factors, with higher incidence in pregnant women, neonates, elderly adults, and individuals with cell-mediated immunodeficiencies. Localized and focal infections can be caused by either direct inoculation of *Listeria* into a site or via hematogenous spread.

Acute Febrile Gastroenteritis

L. monocytogenes been recognized as a cause of outbreaks of febrile gastroenteritis only in the past few decades. The first fully evaluated outbreak happened in 1994, when 45 people developed febrile gastroenteritis after drinking pasteurized chocolate milk contaminated after pasteurization and stored in conditions that allowed for bacterial proliferation.[54] *Listeria* strains from stool cultures, the chocolate milk, and the milk storage tank were indistinguishable, providing for the first time the most compelling evidence for *L. monocytogenes* as an etiologic agent of gastroenteritis.[54] Multiple other outbreaks have since been reported including one directly linking the epidemic strain to subclinical mastitis in a goat whose milk was used to produce fresh cheese.[55] Serotypes 1/2a and 1/2b are responsible for most cases of febrile gastroenteritis, whereas serotype 4b more commonly causes invasive diseases. High

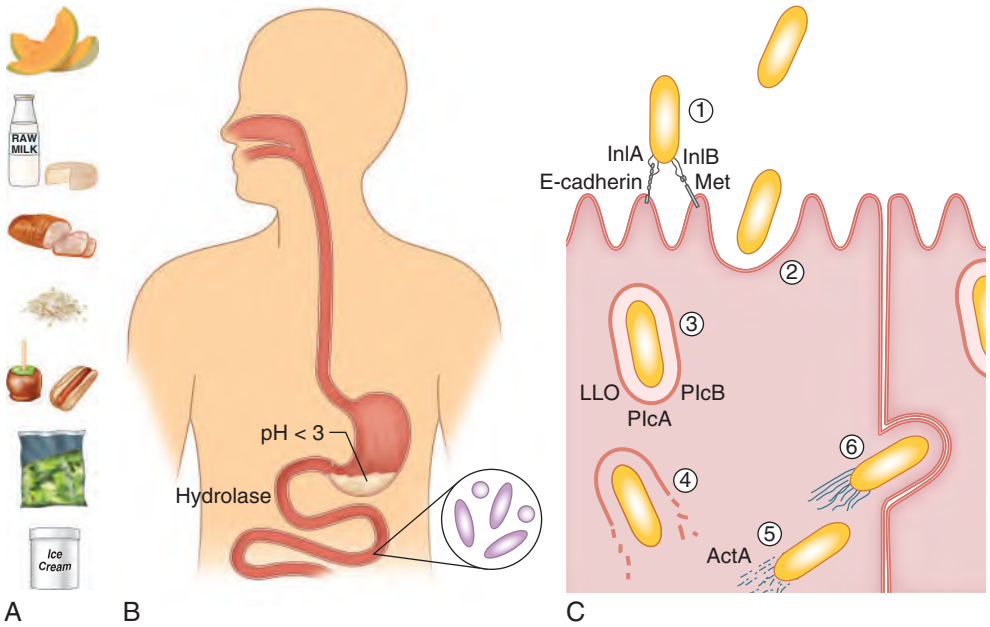


FIG. 206.1 (A) Foods associated with listeriosis outbreaks—cantaloupe, raw dairy products, ready-to-eat meats such as deli meat, sprouts, caramel apples, hot dogs, prepackaged salads, and ice cream. (B) Host defenses and transit through the gastrointestinal tract. In the stomach, gastric pH of less than 3 is informally bactericidal. Bacterial survival increases above a pH of 3.5. *Listeria monocytogenes* expresses bile salt hydrolases and is able to tolerate high-salt conditions, enabling it to survive transit through the duodenum. Paneth and epithelial cells in the small intestine produce bactericidal peptides. Intestinal microbiota provides an independent and important first line of defense against *L. monocytogenes* infection as well as indirectly augmenting host defenses. (C) Intracellular invasion. Bacterial virulence factors and surface proteins Internalin A (InlA) and Internalin B (InlB) bind to the surface receptors E-cadherin and Met *(1)* on nonphagocytic host cells and facilitate entry by receptor-mediated endocytosis *(2)*. After internalization, the low pH of the phagosome activates listeriolysin O (LLO), which, along with phospholipases PlcA and PlcB *(3)*, causes pore formation of the organelle wall and inhibits lysosome fusion, allowing the bacterium to escape *(4)*. Bacterial surface protein ActA recruits and binds actin from the host cell to form an actin comet tail at one pole enabling the bacterium to propel itself through the cytoplasm *(5)*. The bacterium then finds the plasma membrane of the infected host cell, in which it is enveloped, extruded from the infected cell, and phagocytosed by the neighboring cell *(6)*.

organism density in the source food was found in many of the reported outbreaks, suggesting that high inoculum is probably required to produce infection. Virulence and host factors also likely play a role but have yet to be elucidated. Attack rates during outbreaks have ranged from 52% to 100%.[56]

In contrast to invasive listeriosis, gastrointestinal listeriosis occurs most frequently in healthy children and adults. Infection usually has a much less severe clinical course than invasive infection and is generally self-limiting. Symptoms last an average of 1 to 3 days but can persist for a week.[56] Fever, headache, fatigue, arthralgias, nausea, and nonbloody diarrhea are common symptoms. The incubation period is short, with symptoms often occurring within 24 hours after ingestion of contaminated food.[56,57] However, there was one case in an outbreak in Sweden in which a person developed symptoms 10 days after ingestion of contaminated fresh cheese.[58] A small number of invasive infections were reported during febrile gastroenteritis outbreaks, but these were usually in people with underlying risk factors for invasive disease such as older age and chemical immunosuppression.[54,58]

Sporadic cases of *Listeria* gastroenteritis are likely uncommon. A 2-year prospective study in Nova Scotia looked at 6785 stool samples submitted for bacterial culture and found only 13 grew *L. monocytogenes,* whereas there were 124 isolates of *Salmonella* spp. and 128 isolates of *Campylobacter* spp.[59] Study participants, when matched with control cases from the cohort that grew either *Salmonella* spp. or *Campylobacter* spp., had a longer duration of diarrhea and were more likely to have underlying gastrointestinal problems such as inflammatory bowel disease, irritable bowel syndrome, rectal carcinoma, and *Clostridioides difficile* (formerly *Clostridium difficile*) colitis in one case.[59] Participants with stool cultures positive for *L. monocytogenes* were less likely to report fever than persons in the control group (17% vs. 54%).

Additionally, stool carriage of *L. monocytogenes* in the general human population is approximately 1% to 5%, with a higher percentage in individuals in households with members who develop invasive listeriosis,[60–62] presenting a potential challenge in interpreting positive stool cultures. However, *L. monocytogenes* should be considered in patients with febrile gastroenteritis and negative testing for other common pathogens.[56]

Cutaneous Listeriosis

Primary cutaneous listeriosis is rare and has been described in otherwise healthy individuals predominantly with occupational or recreational exposures such as cattle farming, large animal veterinary medicine, and, in one reported case, gardening.[63] Infection occurs via direct inoculation and in many documented cases from an infected source, most commonly aborted bovine fetuses.[63,64] A papulopustular rash develops at the site of inoculation 1 to 7 days after exposure. Nearly all rashes are accompanied by fever. Given the mode of transmission, arms and hands are the most frequent site of infection. In nearly all documented cases, patients made a full recovery with only a quarter receiving treatment with antibiotics, suggesting infection is usually self-limited. Secondary cutaneous listeriosis due to disseminated disease has been reported only in neonates (discussed in "Neonatal Infection") and immunosuppressed adults.[65,66]

Invasive Listeriosis

Pregnancy

Women who are pregnant are at a 17-fold greater risk of listeriosis (12 cases/100,000) than the general population (0.7 cases/100,000) and almost 6-fold greater risk than individuals older than 70 years of age (2.1 cases/100,000).[42] Pregnant women represent about one-sixth of invasive listeriosis cases.[17] Although maternal infection is usually mild, vertical transmission to the fetus can cause fetal loss, preterm labor, and neonatal listeriosis, a condition with high morbidity and mortality. In one study, about 20% of pregnancy-related listeriosis cases led to spontaneous abortion or stillbirth.[67] Among pregnancies that did not end in spontaneous abortion or stillbirth, about 68% of neonates became

infected, and the remaining 32% did not become infected. A large prospective study in France found that major fetal or neonatal complications occurred in up to 83% of maternal cases, which is higher than previous retrospective analyses.[4]

Development of listeriosis earlier in pregnancy correlates with poorer fetal outcomes.[4,68] Women are more susceptible to listeriosis in the third trimester, probably due to a decline in cell-mediated immunity during that period.[67,69,70] Additionally, most pregnant women who develop listeriosis have no other underlying risk factors usually associated with listeriosis and are generally healthy.[67] Certain subpopulations, such as Hispanic women in the United States, women of Asian or Afro-Caribbean descent in the United Kingdom, women from the Maghreb, women from sub-Saharan Africa in France, and non–English-speaking women in Australia, are disproportionally represented among cases of maternal listeriosis.[4,17,68,71]

Clinical manifestations occur an average of 23 days (range, 0–67 days) after exposure and are often subtle and nonspecific, with fever being the most common sign in a number of studies.[4,67,72] Other manifestations may be flulike symptoms or obstetric signs and symptoms such as contractions, labor, abnormal fetal heart rate, abdominal pain, diarrhea, vomiting, headache, amnionitis, or septic abortion.[4,67,68] Blood cultures are positive in 43% to 58% of women, and vaginal or cervical cultures are positive in 26% to 34%.[4,67,71] For reasons not yet elucidated, cases of severe disseminated listeriosis and meningitis are rare in pregnant women, even in women with bacteremia.[67]

Neonatal Infection

From the infected mother, *L. monocytogenes* can invade through the placenta leading to severe complications such as chorioamnionitis, spontaneous abortion, stillbirth, preterm labor, and neonatal infection. Congenital listeriosis, which is rare outside of the neonatal period in children, is a cause of meningitis and disseminated bacterial infections in infants 31 days old or less, with *Listeria,* group B *Streptococcus,* and *Escherichia coli* accounting for more than 70% of total cases.[73,74] The incidence and epidemiology of neonatal listeriosis vary geographically. Historically, *L. monocytogenes* has been one of the top causes of severe bacterial infection in the neonatal period. However, there has been an epidemiologic shift in recent decades with listeriosis becoming less common among other bacterial pathogens affecting neonates, especially in the United States.[75–77] Case rates have also significantly decreased from 4.78 cases for the years 1992–95 to 1.31 cases for 2003–13 per 10,000 admissions in the United States.[75] This shift and decrease in incidence may be explained by a number of factors including improved food safety and implementation of antenatal group B *Streptococcus* screening and prophylaxis with penicillin or amoxicillin.[75]

In the past 50 years, the United Kingdom has seen a decrease in mortality from neonatal listeriosis from 35%[5] to 21% to 24%.[71] A 17-year study from Taiwan found a 29% mortality rate.[78] Neonatal listeriosis produces severe disease with a high mortality rate of 20% to 30%, whereas preceding maternal infection may be absent or produce self-limiting mild flulike symptoms or fever with a sepsis syndrome.[67,79,80] Neonatal listeriosis can manifest in three forms: early-onset sepsis syndrome, late-onset meningitis, and, much less commonly, granulomatosis infantiseptica.[69]

Granulomatosis infantiseptica and probably early-onset listeriosis are acquired by transplacental infection in utero. Granulomatosis infantiseptica is a rapidly fatal disseminated infection present at birth with widespread microabscesses and granulomas within the liver and spleen and sometimes on the skin and bacteria seen on Gram stain of the meconium.[67,69]

Early-onset neonatal listeriosis occurs within the first few days of life. It is associated with preterm birth; chorioamnionitis; and diagnosis of one or a combination of bacteremia, pneumonia, or meningitis.[67,79] In one large case series including 6 cases from 3 large hospitals and 94 cases from the literature, 20.2% of neonates had pneumonia; 25.5% had bacteremia; 29.8% had bacteremia and pneumonia; 9.6% had bacteremia and meningitis; 5.3% had meningitis; and 5.3% had bacteremia, meningitis, and pneumonia.[67] Clinical features, which may be present at birth, include respiratory distress, meconium staining, fever, jaundice, lethargy, and maculopapular or papulovesicular rash.[67,71,78]

Late-onset neonatal listeriosis occurs an average of 5 to 14 days after birth. Neonates are usually full term, are healthy at birth, and are born to asymptomatic mothers who have had uncomplicated pregnancies.[67,79] Although mode of transmission remains unclear, inoculation from the vaginal tract or maternal gastrointestinal tract and nosocomial infection[67,79,81–83] are thought to be potential sources. Clinical features are nonspecific and similar to other bacterial pathogens causing bacteremia and meningitis during this period. CNS involvement is more common in this disease than in early-onset listeriosis and is the predominant clinical syndrome.[5,67] Sequelae of neonatal listeriosis can be significant, with 40% of surviving infants having neurologic or neurodevelopmental impairment on follow-up in one case series.[71]

Bacteremia

Bacteremia is the most common cause of invasive listeriosis and can lead to neurolisteriosis as well as other localized infections such as endocarditis and septic arthritis. Most cases are sporadic.[9] Of nonpregnant individuals who develop *L. monocytogenes* bacteremia, 97% have at least one underlying immunodeficiency, either due to one or more medical conditions or due to immunomodulating medications.[4] Specific risk factors include solid-organ and hematologic malignancy, kidney disease, cirrhosis, diabetes mellitus, giant cell arteritis, solid-organ transplantation, acquired immunodeficiency syndrome (AIDS), and older age (generally 65 years old and older).[4,69,84–86] Infection occurs in older adults even without other risk factors, with the incidence in individuals age 75 years and older nearly 20 times greater (0.98 cases/100,000) than in individuals younger than age 65 years (0.05 cases/100,000).[84] Current or recent use of a proton pump inhibitor also increases the risk of developing invasive infection.[35] Three-month mortality is 46% and is associated with older age, female sex, ongoing malignancy, multiple organ failure, worsening of prior organ disease, weight loss, monocytopenia <200 cells/μL, and elevated neutrophil count.[4]

Clinical manifestations occur an average of 5 days after exposure (range, 0–29 days).[72] In a large prospective study of 427 cases of bacteremia, 94% of patients presented with fever, tachycardia, or both, and C-reactive protein was elevated in 96%.[4] Signs and symptoms are general, nonspecific, and similar to other pathogens that cause bacteremia. Patients may report an antecedent diarrheal illness or nausea.

Neurolisteriosis

Neurolisteriosis is the second most common manifestation of invasive listeriosis after bacteremia. In the past 2 decades the overall incidence of bacterial meningitis including *L. monocytogenes* has declined to 0.7 to 0.9/100,000 cases in the Western world due to vaccines for *Streptococcus pneumoniae, Neisseria meningitidis,* and *H. influenzae* as well as improved food-processing techniques, safer food practices, and education.[87] Incidence in the developing world remains high.[87,88] In the United States the incidence of *L. monocytogenes* meningitis has declined by 46% over 2 decades from 0.10 cases/100,000 in 1998–99 to 0.05 cases/100,000 in 2006–07.[89] However, *L. monocytogenes* remains one of the top five most common pathogens of CNS infections in the Western world; is third worldwide; and is second among patients with diabetes, with alcohol dependence, and on immunosuppressive therapies.[87,89,90] Despite this overall decline, case mortality rates of neurolisteriosis remain the same, and compared with other pathogens causing CNS infections, *L. monocytogenes* mortality continues to be among the highest.[89]

In contrast to other common pathogens that cause bacterial meningitis, *L. monocytogenes* has a proclivity for the brain itself.[69] Isolated meningitis (13%) is less common than meningoencephalitis (84%).[4] *L. monocytogenes* can also cause localized infections such as cerebritis; abscess; and rhombencephalitis, a rare form of encephalitis involving the cerebellum and brainstem. The presence of encephalitis is associated with older age and increased number of comorbidities and a threefold greater mortality compared with neurolisteriosis without encephalitis.[4]

Although the same risk factors exist for individuals who develop *Listeria* bacteremia as individuals who develop CNS disease, 14% to 37% of persons who develop neurolisteriosis have no identifiable risk factors.[4,91,92] A large prospective study in France found 4% of patients with neurolisteriosis were younger than 40 years old, excluding neonates,

and had no identifiable risk factors including pregnancy.[4] This suggests a possible genetic susceptibility in this small subset of patients or perhaps exposure to a higher bacterial inoculum.[4] Individuals without underlying disease or other risk factors have a lower mortality rate than individuals with existing risk factors. Mortality rates range from 9% to 38% in this previously healthy group and higher depending on geographic location.[4,69,91,93]

The average incubation period is 10 days (range, 0–21 days).[72] Fever, headache, myalgia, chills, gastroenteritis, and other systemic symptoms may occur an average of 3 to 4 days before presentation.[91] Fever is the most common presenting symptom (>90%),[4,94] followed by altered mental status (66%) and headache (46%).[4,91] Seizures, cranial neuropathies, and other focal neuropathies may also be present. One-half to two-thirds of patients have nuchal rigidity.[4,91]

Gram stain of CSF reveals gram-positive rods in only about one-third of cases.[4,91,94] CSF analysis usually reveals a pleocytosis with neutrophil predominance. Blood cultures are positive in more than half of patients. In one small study, hyponatremia was present in 22 of 30 (73%) cases.[94] In a large prospective study, in a subanalysis of neuroradiographic findings in 71 patients with neurolisteriosis, magnetic resonance imaging was more sensitive than computed tomography for showing meningeal and parenchymal involvement of the brain.[4]

Three-month mortality is 30% and similar to patients with bacteremia without CNS involvement. Factors associated with mortality include older age, female sex, ongoing malignancy, multiple organ failure, worsening of prior organ disease, weight loss, monocytopenia <200 cells/μL, elevated neutrophil count, bacteremia, and dexamethasone administration.[4]

Of patients who survive, about 40% recover fully, and about 45% have persistent neurologic impairment such as limb motor deficiencies, cerebellar symptoms, and eighth nerve palsy.[4] Encephalitis is the strongest predictor of persistent neurologic impairment.[4]

Focal Invasive Infection

L. monocytogenes can cause endocarditis,[95] endovascular infections,[96] septic arthritis,[97,98] osteomyelitis,[98] peritonitis,[99] adenitis,[4] urinary tract infections,[100] pneumonia,[101] and other focal infections via hematogenous spread. Focal infections can also precede systemic infection.[102] Ocular infections can occur as a result of either direct inoculation or hematogenous spread.[103–105]

DIAGNOSIS

Listeriosis should be particularly suspected in the following clinical scenarios: respiratory distress, sepsis, or meningitis in neonates; meningitis or parenchymal brain infection in persons ≥50 years of age with subacute presentation of meningitis, hematologic malignancy, solid-organ malignancy, organ transplant, or AIDS or on immunosuppressive therapies (e.g., chemotherapy for cancer, corticosteroids, anti-TNF agents); concomitant infection of the meninges and brain parenchyma; subcortical brain abscess; fever during pregnancy, especially in the third trimester; and foodborne outbreak of febrile gastroenteritis with negative stool cultures or standard gastrointestinal panel multiplex PCR.[106]

Diagnosis of invasive listeriosis is usually made by isolation of *L. monocytogenes* by culture from sterile sources such as blood, CSF, amniotic fluid, placental tissue, aqueous humor, or vitreous fluid. Culture from peripheral sites such as the vagina, cervix, and stool in adults and gastric aspirate, ear, anus, and pharynx in neonates can aid in diagnosis.[4] Neurolisteriosis may be diagnosed in patients with positive blood cultures and neurologic symptoms consistent with meningitis, encephalitis, or cerebritis without positive CSF cultures. Cultures may be negative if patients received empirical antimicrobial treatment before sample collection. PCR is more sensitive and more specific than culture, offers rapid diagnosis, and can be used in cases to confirm diagnoses when cultures are negative but clinical suspicion is high.[107] Multiplex PCR testing for CSF for an array of viruses, bacteria including *L. monocytogenes,* and fungi is now commercially available.[108]

Patients usually have fever and a leukocytosis with predominance of polymorphonuclear cells.[4] In nonperinatal cases, 79% to 84% of patients have lymphopenia, but this is less common in maternal cases.[4] Despite its name, *L. monocytogenes* infections rarely produce a monocytosis in humans.[4,69]

Isolation of *L. monocytogenes* from wound and urine cultures can be due to contamination, colonization, or true infection. Results should be interpreted alongside other tests such as urinalysis as well as within the clinical context from which the culture was obtained.[100] Selective media are required for isolation from stool, and the receiving microbiology laboratory should be notified if *Listeria* gastroenteritis is suspected.[56] Although not useful in invasive disease, elevated levels of LLO antibodies may be helpful in the diagnosis of febrile gastroenteritis.[54,106]

TREATMENT

Although there are no randomized controlled trials to establish the most effective antimicrobial treatment of listeriosis, benzylpenicillin (penicillin G) and aminopenicillin (ampicillin) are considered the mainstay of treatment either alone or in combination with an aminoglycoside for synergy in severe infections such as CNS infections or endocarditis.[109] Second-line therapies include trimethoprim-sulfamethoxazole, meropenem, and piperacillin-tazobactam, although the latter should not be used in CNS infections. Cephalosporins should not be used.[69,110]

In vitro and extracellular susceptibilities of clinical isolates of *L. monocytogenes* demonstrate sensitivity to many antibiotics including penicillin, ampicillin, fluoroquinolones, vancomycin, tetracycline, rifampin, and cefazolin with high resistance rates to clindamycin and third-generation cephalosporins.[111] Intracellular activity may vary,[112] and there have been clinical failures with vancomycin and development of listeriosis while on ciprofloxacin.[113,114] In rare instances, plasmid-mediated resistance to macrolides, gentamicin, and trimethoprim occurs.[115]

In in vitro studies, β-lactams are bacteriostatic for *L. monocytogenes,* and the addition of an aminoglycoside has been shown to synergistically enhance killing.[110] However, there are mixed data with regard to benefit of aminoglycosides in both animal and clinical studies given their toxicity. A number of retrospective studies did not show improved outcomes with aminoglycoside synergy, and one showed higher mortality with aminoglycoside use.[116–118] Other large studies including a more recent prospective study showed statistically significant increased survival.[4,91] Consequently, both US and European guidelines for the treatment of *L. monocytogenes* meningitis recommend considering the addition of an aminoglycoside rather than a formal recommendation for combination therapy.[119,120]

Local infections such as cutaneous and febrile gastroenteritis, which occur mainly in immunocompetent hosts, are often self-limited and resolve without antibiotics.[63,121] Pregnant women with febrile gastroenteritis and presumptive exposure to *L. monocytogenes* should be treated with ampicillin or amoxicillin.[122,123] Otherwise, antibiotics may be considered in protracted cases or cases with underlying risk factors for severe infection.

The optimal duration of treatment for invasive listeriosis is unknown. Patients with pregnancy-associated listeriosis and bacteremia should receive at least 2 weeks of antimicrobial treatment.[124] Trimethoprim-sulfamethoxazole should be avoided in women in the first trimester of pregnancy, as it can cause neural tube and cardiovascular defects.[123]

In neonates, individuals with predisposing risk factors, and adults older than 50 years of age, empirical treatment for bacterial meningitis should include an anti-*Listeria* agent.[119,120] *Listeria* meningitis should be treated for at least 21 days.[119,120] Treatment of focal infections such as brain abscess may require treatment for 6 to 8 weeks.[69,91] Adjunctive treatment for bacterial meningitis with steroids, which have been shown to improve mortality in cases of *S. pneumoniae* meningitis,[125] should be stopped once *L. monocytogenes* is identified as the causative pathogen because steroid therapy is associated with increased mortality in this subset of patients.[4]

PREVENTION

Pneumocystis prophylaxis with trimethoprim-sulfamethoxazole likely also protects against listeriosis, but the incidence of listeriosis in the same populations is too low to evaluate efficacy. Prevention of listeriosis requires a multipronged approach including regulation and testing at the food-processing level as well as education of high-risk populations on safe food consumption and handling practices.

Since 1987, the US Department of Agriculture Food Safety and Inspection Service has implemented on-site testing for *L. monocytogenes* at meat and poultry plants and random sampling programs and prohibited

TABLE 206.1 Dietary Recommendations for Preventing Foodborne Listeriosis

General Recommendations
Washing and Handling Food
• Rinse raw produce thoroughly under running tap water before eating, cutting, or cooking. Even if produce will be peeled, it should still be washed first. • Scrub firm produce, such as melons and cucumbers, with a clean produce brush. • Dry produce with a clean cloth or paper towel.
Keep the Kitchen Cleaner and Safer
• Wash hands, knives, countertops, and cutting boards after handling and preparing uncooked foods. • Be aware that *Listeria monocytogenes* can grow in foods in the refrigerator. The refrigerator should be 40°F or lower, and the freezer should be 0°F or lower. • Clean up all spills in the refrigerator right away, especially juices from hot dog and lunchmeat packages, raw meat, and raw poultry.
Cook Meat and Poultry Thoroughly
• Thoroughly cook raw food from animal sources such as beef, pork, or poultry to a safe internal temperature. • Use precooked or ready-to-eat food as soon as you can. Do not store the product in the refrigerator beyond the use-by date. • Use leftovers within 3–4 days.
Choose Safer Foods
• Do not drink raw (unpasteurized) milk, and do not eat foods that have unpasteurized milk in them.
Recommendations for Persons at Higher Risk[a]
Meats
• Do not eat hot dogs, luncheon meats, cold cuts, other delicatessen meats (e.g., bologna), or fermented or dry sausages unless they are heated to an internal temperature of 165°F or until steaming hot just before serving. • Avoid getting fluid from hot dog and lunchmeat packages on other foods, utensils, and food preparation surfaces, and wash hands after handling hot dogs, luncheon meats, and delicatessen meats. • Do not eat refrigerated pâté or meat spreads from a delicatessen or meat counter or from the refrigerated section of a store. Foods that do not need refrigeration, such as canned or shelf-stable pâté and meat spreads, are safe to eat. Refrigerate after opening.
Cheeses
• Do not eat soft cheese such as feta, queso blanco, queso fresco, brie, Camembert, blue-veined, or panela (queso panela) unless it is labeled as "*Made With Pasteurized Milk*."
Seafood
• Do not eat refrigerated smoked seafood unless it is contained in a cooked dish, such as a casserole, or unless it is a canned or shelf-stable product. • Canned and shelf-stable tuna, salmon, and other fish products are safe to eat.
Melons
• Wash hands with warm water and soap for at least 20 seconds before and after handling any whole melon. • Scrub surface of melons with a clean produce brush under running water and dry them with a clean cloth or paper towel before cutting. Be sure that your scrub brush is sanitized after each use. • Promptly consume cut melon or refrigerate promptly. Keep cut melon refrigerated for no more than 7 days. • Discard cut melons left at room temperature for more than 4 hours.
Raw Sprouts
• Do not eat any kind of raw or lightly cooked sprouts and ask that raw or lightly cooked sprouts not be added to food when eating out. • Cooking sprouts thoroughly can reduce the risk of getting sick.

[a]Recommendations for persons at higher risk such as pregnant women, persons with weakened immune systems, and older adults are in addition to the recommendations listed under General Recommendations.

Modified from Lorber B. Listeria monocytogenes. In: Bennett JE, Dolin R, Blaser MJ, eds. Principles and Practice of Infectious Diseases. *8th ed. Philadelphia: Saunders; 2015.*

the selling of contaminated foods.[9,16] These policies, guidelines, and regulations, along with better treatments for human immunodeficiency virus and education of high-risk groups, have contributed to a decrease in incidence of sporadic cases of listeriosis as well as a decrease in mortality annually in the United States of 10.74% for the years 1990–96 to 4.29% for the years 1996–2005.[16] The development of surveillance and reporting systems in the United States in the late 1990s and early 2000s has improved and expedited detection of outbreaks resulting in smaller and shorter outbreaks.[9] Additionally, these systems have resulted in a decrease in incidence overall since implementation, although for the years 2001–09 the number of cases of invasive disease plateaued with the exception of a peak in 2011 that was due to a large cantaloupe-associated outbreak.[9,13,17,126] In Europe a decrease in incidence was seen for the years 1999–2005, but overall incidence increased in 2006 due to an increase in sporadic cases of bacteremia in individuals older than 60 years of age and individuals younger than 60 with underlying risk factors such as leukemia.[18]

High-risk populations often report consumption of foods associated with listeriosis and should be educated on the risks of listeriosis and advised to avoid foods implicated in previous outbreaks.[17] Recognition and education of subset populations at even higher risk such as pregnant Hispanic women in the United States should be prioritized.[9,17] Recommendations for safer food handling practices at home are summarized in Table 206.1.

Key References

The complete reference list is available online at Expert Consult.

3. Schlech WF 3rd, Lavigne PM, Bortolussi RA, et al. Epidemic listeriosis—evidence for transmission by food. *N Engl J Med*. 1983;308:203–206.
4. Charlier C, Perrodeau E, Leclercq A, et al. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. *Lancet Infect Dis*. 2017;17:510–519.
7. de Noordhout CM, Devleesschauwer B, Angulo FJ, et al. The global burden of listeriosis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2014;14:1073–1082.
8. Varma JK, Samuel MC, Marcus R, et al. *Listeria monocytogenes* infection from foods prepared in a commercial establishment: a case-control study of potential sources of sporadic illness in the United States. *Clin Infect Dis*. 2007;44:521–528.
9. Cartwright EJ, Jackson KA, Johnson SD, et al. Listeriosis outbreaks and associated food vehicles, United States, 1998-2008. *Emerg Infect Dis*. 2013;19:1–9, quiz 184.
13. McCollum JT, Cronquist AB, Silk BJ, et al. Multistate outbreak of listeriosis associated with cantaloupe. *N Engl J Med*. 2013;369:944–953.

14. Buchanan RL, Gorris LGM, Hayman MM, et al. A review of *Listeria monocytogenes*: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control*. 2017;75:1–13.
15. Rocourt J, BenEmbarek P, Toyofuku H, et al. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat foods: the FAO/WHO approach. *FEMS Immunol Med Microbiol*. 2003;35:263–267.
16. Bennion JR, Sorvillo F, Wise ME, et al. Decreasing listeriosis mortality in the United States, 1990-2005. *Clin Infect Dis*. 2008;47:867–874.
17. Silk BJ, Date KA, Jackson KA, et al. Invasive listeriosis in the Foodborne Diseases Active Surveillance Network (FoodNet), 2004-2009: further targeted prevention needed for higher-risk groups. *Clin Infect Dis*. 2012;54:S396–S404.
18. Goulet V, Hedberg C, Le Monnier A, et al. Increasing incidence of listeriosis in France and other European countries. *Emerg Infect Dis*. 2008;14:734–740.
30. Freitag NE, Port GC, Miner MD. *Listeria monocytogenes*—from saprophyte to intracellular pathogen. *Nat Rev Microbiol*. 2009;7:623–628.
32. Mostowy S, Cossart P. Virulence factors that modulate the cell biology of *Listeria* infection and the host response. *Adv Immunol*. 2012;113:19–32.
35. Kvistholm Jensen A, Simonsen J, Ethelberg S. Use of proton pump inhibitors and the risk of listeriosis: a nationwide registry-based case-control study. *Clin Infect Dis*. 2017;64:845–851.
37. Becattini S, Pamer EG. Multifaceted defense against *Listeria monocytogenes* in the gastro-intestinal lumen. *Pathogens*. 2017;7.
39. Becattini S, Littmann ER, Carter RA, et al. Commensal microbes provide first line defense against *Listeria monocytogenes* infection. *J Exp Med*. 2017;214:1973–1989.
40. Quereda JJ, Dussurget O, Nahori MA, et al. Bacteriocin from epidemic *Listeria* strains alters the host intestinal microbiota to favor infection. *Proc Natl Acad Sci USA*. 2016;113:5706–5711.
41. Radoshevich L, Cossart P. *Listeria monocytogenes*: towards a complete picture of its physiology and pathogenesis. *Nat Rev Microbiol*. 2018;16:32–46.
44. Nikitas G, Deschamps C, Disson O, et al. Transcytosis of *Listeria monocytogenes* across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin. *J Exp Med*. 2011;208:2263–2277.
49. Sherrid AM, Kollmann TR. Age-dependent differences in systemic and cell-autonomous immunity to *L. monocytogenes*. *Clin Dev Immunol*. 2013;2013:917198.
50. Pamer EG. Immune responses to *Listeria monocytogenes*. *Nat Rev Immunol*. 2004;4:812–823.
51. Witte CE, Archer KA, Rae CS, et al. Innate immune pathways triggered by *Listeria monocytogenes* and their role in the induction of cell-mediated immunity. *Adv Immunol*. 2012;113:135–156.
53. Zenewicz LA, Shen H. Innate and adaptive immune responses to *Listeria monocytogenes*: a short overview. *Microbes Infect*. 2007;9:1208–1215.
54. Dalton CB, Austin CC, Sobel J, et al. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. *N Engl J Med*. 1997;336:100–105.
55. Ooi ST, Lorber B. Gastroenteritis due to *Listeria monocytogenes*. *Clin Infect Dis*. 2005;40:1327–1332.
57. Goulet V, King LA, Vaillant V, et al. What is the incubation period for listeriosis? *BMC Infect Dis*. 2013;13:11.
59. Schlech WF 3rd, Schlech WF 4th, Haldane H, et al. Does sporadic *Listeria* gastroenteritis exist? A 2-year population-based survey in Nova Scotia, Canada. *Clin Infect Dis*. 2005;41:778–784.
63. Godshall CE, Suh G, Lorber B. Cutaneous listeriosis. *J Clin Microbiol*. 2013;51:3591–3596.
67. Mylonakis E, Paliou M, Hohmann EL, et al. Listeriosis during pregnancy: a case series and review of 222 cases. *Medicine (Baltimore)*. 2002;81:260–269.
69. Lorber B. Listeriosis. *Clin Infect Dis*. 1997;24:1–9, quiz 10–11.
72. Angelo KM, Jackson KA, Wong KK, et al. Assessment of the incubation period for invasive listeriosis. *Clin Infect Dis*. 2016;63:1487–1489.
76. Schrag SJ, Farley MM, Petit S, et al. Epidemiology of invasive early-onset neonatal sepsis, 2005 to 2014. *Pediatrics*. 2016;138.
79. Lamont RF, Sobel J, Mazaki-Tovi S, et al. Listeriosis in human pregnancy: a systematic review. *J Perinat Med*. 2011;39:227–236.
84. Goulet V, Hebert M, Hedberg C, et al. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clin Infect Dis*. 2012;54:652–660.
85. Fernandez-Sabe N, Cervera C, Lopez-Medrano F, et al. Risk factors, clinical features, and outcomes of listeriosis in solid-organ transplant recipients: a matched case-control study. *Clin Infect Dis*. 2009;49:1153–1159.
86. Guevara RE, Mascola L, Sorvillo F. Risk factors for mortality among patients with nonperinatal listeriosis in Los Angeles County, 1992-2004. *Clin Infect Dis*. 2009;48:1507–1515.
91. Mylonakis E, Hohmann EL, Calderwood SB. Central nervous system infection with *Listeria monocytogenes*. 33 years' experience at a general hospital and review of 776 episodes from the literature. *Medicine (Baltimore)*. 1998;77:313–336.
92. Maertens De Noordhout C, Devleesschauwer B, Maertens De Noordhout A, et al. Comorbidities and factors associated with central nervous system infections and death in non-perinatal listeriosis: a clinical case series. *BMC Infect Dis*. 2016;16:256.
93. Skogberg K, Syrjanen J, Jahkola M, et al. Clinical presentation and outcome of listeriosis in patients with and without immunosuppressive therapy. *Clin Infect Dis*. 1992;14:815–821.
94. Brouwer MC, van de Beek D, Heckenberg SG, et al. Community-acquired *Listeria monocytogenes* meningitis in adults. *Clin Infect Dis*. 2006;43:1233–1238.
109. Thonnings S, Knudsen JD, Schonheyder HC, et al. Antibiotic treatment and mortality in patients with *Listeria monocytogenes* meningitis or bacteraemia. *Clin Microbiol Infect*. 2016;22:725–730.
112. Seral C, Barcia-Macay M, Mingeot-Leclercq MP, et al. Comparative activity of quinolones (ciprofloxacin, levofloxacin, moxifloxacin and garenoxacin) against extracellular and intracellular infection by *Listeria monocytogenes* and *Staphylococcus aureus* in J774 macrophages. *J Antimicrob Chemother*. 2005;55:511–517.
117. Arslan F, Meynet E, Sunbul M, et al. The clinical features, diagnosis, treatment, and prognosis of neuroinvasive listeriosis: a multinational study. *Eur J Clin Microbiol Infect Dis*. 2015;34:1213–1221.
118. Amaya-Villar R, Garcia-Cabrera E, Sulleiro-Igual E, et al. Three-year multicenter surveillance of community-acquired *Listeria monocytogenes* meningitis in adults. *BMC Infect Dis*. 2010;10:324.
121. Olsen SJ, Patrick M, Hunter SB, et al. Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clin Infect Dis*. 2005;40:962–967.

References

1. Murray EGD, Webb RA, Swann MBR. A disease of rabbits characterised by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp.). *J Pathol Bacteriol*. 1926;29: 1555–2039.
2. Gray ML, Killinger AH. Listeria monocytogenes and listeric infections. *Bacteriol Rev*. 1966;30:309–382.
3. Schlech WF 3rd, Lavigne PM, Bortolussi RA, et al. Epidemic listeriosis—evidence for transmission by food. *N Engl J Med*. 1983;308:203–206.
4. Charlier C, Perrodeau E, Leclercq A, et al. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. *Lancet Infect Dis*. 2017;17:510–519.
5. McLauchlin J. Human listeriosis in Britain, 1967-85, a summary of 722 cases. 2. Listeriosis in non-pregnant individuals, a changing pattern of infection and seasonal incidence. *Epidemiol Infect*. 1990;104:191–201.
6. Scallan E, Hoekstra RM, Mahon BE, et al. An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiol Infect*. 2015;143:2795–2804.
7. de Noordhout CM, Devleesschauwer B, Angulo FJ, et al. The global burden of listeriosis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2014;14:1073–1082.
8. Varma JK, Samuel MC, Marcus R, et al. Listeria monocytogenes infection from foods prepared in a commercial establishment: a case-control study of potential sources of sporadic illness in the United States. *Clin Infect Dis*. 2007;44:521–528.
9. Cartwright EJ, Jackson KA, Johnson SD, et al. Listeriosis outbreaks and associated food vehicles, United States, 1998-2008. *Emerg Infect Dis*. 2013;19:1–9, quiz 184.
10. ILSI Research Foundation; Risk Science Institute. Achieving continuous improvement in reductions in foodborne listeriosis—a risk-based approach. *J Food Prot*. 2005;68:1932–1994.
11. Swaminathan B, Gerner-Smidt P. The epidemiology of human listeriosis. *Microbes Infect*. 2007;9:1236–1243.
12. Listeria Outbreaks | Listeria | CDC. 2017.
13. McCollum JT, Cronquist AB, Silk BJ, et al. Multistate outbreak of listeriosis associated with cantaloupe. *N Engl J Med*. 2013;369:944–953.
14. Buchanan RL, Gorris LGM, Hayman MM, et al. A review of *Listeria monocytogenes*: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control*. 2017;75:1–13.
15. Rocourt J, BenEmbarek P, Toyofuku H, et al. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat foods: the FAO/WHO approach. *FEMS Immunol Med Microbiol*. 2003;35:263–267.
16. Bennion JR, Sorvillo F, Wise ME, et al. Decreasing listeriosis mortality in the United States, 1990-2005. *Clin Infect Dis*. 2008;47:867–874.
17. Silk BJ, Date KA, Jackson KA, et al. Invasive listeriosis in the Foodborne Diseases Active Surveillance Network (FoodNet), 2004-2009: further targeted prevention needed for higher-risk groups. *Clin Infect Dis*. 2012;54:S396–S404.
18. Goulet V, Hedberg C, Le Monnier A, et al. Increasing incidence of listeriosis in France and other European countries. *Emerg Infect Dis*. 2008;14:734–740.
19. Orsi RH, Wiedmann M. Characteristics and distribution of *Listeria* spp., including *Listeria* species newly described since 2009. *Appl Microbiol Biotechnol*. 2016;100: 5273–5287.
20. Guillet C, Join-Lambert O, Le Monnier A, et al. Human listeriosis caused by *Listeria ivanovii*. *Emerg Infect Dis*. 2010;16:136–138.
21. Rapose A, Lick SD, Ismail N. *Listeria grayi* bacteremia in a heart transplant recipient. *Transpl Infect Dis*. 2008;10:434–436.
22. Salimnia H, Patel D, Lephart PR, et al. *Listeria grayi*: vancomycin-resistant, gram-positive rod causing bacteremia in a stem cell transplant recipient. *Transpl Infect Dis*. 2010;12:526–528.
23. Allerberger F. *Listeria*: growth, phenotypic differentiation and molecular microbiology. *FEMS Immunol Med Microbiol*. 2003;35:183–189.
24. Burall LS, Laksanalamai P, Datta AR. *Listeria monocytogenes* mutants with altered growth phenotypes at refrigeration temperature and high salt concentrations. *Appl Environ Microbiol*. 2012;78:1265–1272.
25. Buchner LH, Schneierson S. Clinical and laboratory aspects of *Listeria monocytogenes* infections. With a report of ten cases. *Am J Med*. 1968;45:904–921.
26. Curtis GD, Lee WH. Culture media and methods for the isolation of *Listeria monocytogenes*. *Int J Food Microbiol*. 1995;26:1–13.
27. Beumer RR, Hazeleger WC. *Listeria monocytogenes*: diagnostic problems. *FEMS Immunol Med Microbiol*. 2003;35:191–197.
28. Borucki MK, Call DR. *Listeria monocytogenes* serotype identification by PCR. *J Clin Microbiol*. 2003;41:5537–5540.
29. Nho SW, Abdelhamed H, Reddy S, et al. Identification of high-risk *Listeria monocytogenes* serotypes in lineage I (serotype 1/2a, 1/2c, 3a and 3c) using multiplex PCR. *J Appl Microbiol*. 2015;119:845–852.
30. Freitag NE, Port GC, Miner MD. *Listeria monocytogenes*—from saprophyte to intracellular pathogen. *Nat Rev Microbiol*. 2009;7:623–628.
31. Chatterjee SS, Hossain H, Otten S, et al. Intracellular gene expression profile of *Listeria monocytogenes*. *Infect Immun*. 2006;74:1323–1338.
32. Mostowy S, Cossart P. Virulence factors that modulate the cell biology of *Listeria* infection and the host response. *Adv Immunol*. 2012;113:19–32.
33. Kumar A, Grover S, Batish VK. Exploring specific primers targeted against different genes for a multiplex PCR for detection of *Listeria monocytogenes*. *3 Biotech*. 2015;5:261–269.
34. Jiang L, Olesen I, Andersen T, et al. Survival of *Listeria monocytogenes* in simulated gastrointestinal system and transcriptional profiling of stress- and adhesion-related genes. *Foodborne Pathog Dis*. 2010;7:267–274.
35. Kvistholm Jensen A, Simonsen J, Ethelberg S. Use of proton pump inhibitors and the risk of listeriosis: a nationwide registry-based case-control study. *Clin Infect Dis*. 2017;64:845–851.
36. Hofmann AF, Eckmann L. How bile acids confer gut mucosal protection against bacteria. *Proc Natl Acad Sci USA*. 2006;103:4333–4334.
37. Becattini S, Pamer EG. Multifaceted defense against *Listeria monocytogenes* in the gastro-intestinal lumen. *Pathogens*. 2017;7.
38. Dowd GC, Joyce SA, Hill C, et al. Investigation of the mechanisms by which *Listeria monocytogenes* grows in porcine gallbladder bile. *Infect Immun*. 2011;79:369–379.
39. Becattini S, Littmann ER, Carter RA, et al. Commensal microbes provide first line defense against *Listeria monocytogenes* infection. *J Exp Med*. 2017;214:1973–1989.
40. Quereda JJ, Dussurget O, Nahori MA, et al. Bacteriocin from epidemic *Listeria* strains alters the host intestinal microbiota to favor infection. *Proc Natl Acad Sci USA*. 2016;113:5706–5711.
41. Radoshevich L, Cossart P. *Listeria monocytogenes*: towards a complete picture of its physiology and pathogenesis. *Nat Rev Microbiol*. 2018;16:32–46.
42. Southwick FS, Purich DL. Intracellular pathogenesis of listeriosis. *N Engl J Med*. 1996;334:770–776.
43. Lambrechts A, Gevaert K, Cossart P, et al. *Listeria* comet tails: the actin-based motility machinery at work. *Trends Cell Biol*. 2008;18:220–227.
44. Nikitas G, Deschamps C, Disson O, et al. Transcytosis of *Listeria monocytogenes* across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin. *J Exp Med*. 2011;208:2263–2277.
45. Drevets DA, Bronze MS. *Listeria monocytogenes*: epidemiology, human disease, and mechanisms of brain invasion. *FEMS Immunol Med Microbiol*. 2008;53:151–165.
46. Pizarro-Cerda J, Kuhbacher A, Cossart P. Entry of *Listeria monocytogenes* in mammalian epithelial cells: an updated view. *Cold Spring Harb Perspect Med*. 2012;2.
47. Vazquez-Boland JA, Krypotou E, Scortti M. *Listeria* placental infection. *MBio*. 2017;8.
48. Faralla C, Rizzuto GA, Lowe DE, et al. InlP, a new virulence factor with strong placental tropism. *Infect Immun*. 2016;84:3584–3596.
49. Sherrid AM, Kollmann TR. Age-dependent differences in systemic and cell-autonomous immunity to *L. monocytogenes*. *Clin Dev Immunol*. 2013;2013:917198.
50. Pamer EG. Immune responses to *Listeria monocytogenes*. *Nat Rev Immunol*. 2004;4:812–823.
51. Witte CE, Archer KA, Rae CS, et al. Innate immune pathways triggered by *Listeria monocytogenes* and their role in the induction of cell-mediated immunity. *Adv Immunol*. 2012;113:135–156.
52. Rogers HW, Unanue ER. Neutrophils are involved in acute, nonspecific resistance to *Listeria monocytogenes* in mice. *Infect Immun*. 1993;61:5090–5096.
53. Zenewicz LA, Shen H. Innate and adaptive immune responses to *Listeria monocytogenes*: a short overview. *Microbes Infect*. 2007;9:1208–1215.
54. Dalton CB, Austin CC, Sobel J, et al. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. *N Engl J Med*. 1997;336:100–105.
55. Danielsson-Tham ML, Eriksson E, Helmersson S, et al. Causes behind a human cheese-borne outbreak of gastrointestinal listeriosis. *Foodborne Pathog Dis*. 2004;1:153–159.
56. Ooi ST, Lorber B. Gastroenteritis due to *Listeria monocytogenes*. *Clin Infect Dis*. 2005;40:1327–1332.
57. Goulet V, King LA, Vaillant V, et al. What is the incubation period for listeriosis? *BMC Infect Dis*. 2013;13:11.
58. Carrique-Mas JJ, Hokeberg I, Andersson Y, et al. Febrile gastroenteritis after eating on-farm manufactured fresh cheese—an outbreak of listeriosis? *Epidemiol Infect*. 2003;130:79–86.
59. Schlech WF 3rd, Schlech WF 4th, Haldane H, et al. Does sporadic *Listeria* gastroenteritis exist? A 2-year population-based survey in Nova Scotia, Canada. *Clin Infect Dis*. 2005;41:778–784.
60. Hof H. Listeria monocytogenes: a causative agent of gastroenteritis? *Eur J Clin Microbiol Infect Dis*. 2001;20:369–373.
61. Grif K, Patscheider G, Dierich MP, et al. Incidence of fecal carriage of *Listeria monocytogenes* in three healthy volunteers: a one-year prospective stool survey. *Eur J Clin Microbiol Infect Dis*. 2003;22:16–20.
62. Schuchat A, Deaver K, Hayes PS, et al. Gastrointestinal carriage of *Listeria monocytogenes* in household contacts of patients with listeriosis. *J Infect Dis*. 1993;167:1261–1262.
63. Godshall CE, Suh G, Lorber B. Cutaneous listeriosis. *J Clin Microbiol*. 2013;51:3591–3596.
64. Zelenik K, Avbersek J, Pate M, et al. Cutaneous listeriosis in a veterinarian with the evidence of zoonotic transmission—a case report. *Zoonoses Public Health*. 2014;61:238–241.
65. Lambotte O, Fihman V, Poyart C, et al. *Listeria monocytogenes* skin infection with cerebritis and haemophagocytosis syndrome in a bone marrow transplant recipient. *J Infect*. 2005;50:356–358.
66. Salata RA, King RE, Gose F, et al. *Listeria monocytogenes* cerebritis, bacteremia, and cutaneous lesions complicating hairy cell leukemia. *Am J Med*. 1986;81:1068–1072.
67. Mylonakis E, Paliou M, Hohmann EL, et al. Listeriosis during pregnancy: a case series and review of 222 cases. *Medicine (Baltimore)*. 2002;81:260–269.
68. Elinav H, Hershko-Klement A, Valinsky L, et al. Pregnancy-associated listeriosis: clinical characteristics and geospatial analysis of a 10-year period in Israel. *Clin Infect Dis*. 2014;59:953–961.
69. Lorber B. Listeriosis. *Clin Infect Dis*. 1997;24:1–9, quiz 10–11.
70. Weinberg ED. Pregnancy-associated depression of cell-mediated immunity. *Rev Infect Dis*. 1984;6:814–831.
71. Sapuan S, Kortsalioudaki C, Anthony M, et al. Neonatal listeriosis in the UK 2004-2014. *J Infect*. 2017;74:236–242.
72. Angelo KM, Jackson KA, Wong KK, et al. Assessment of the incubation period for invasive listeriosis. *Clin Infect Dis*. 2016;63:1487–1489.
73. Stoll BJ, Hansen NI, Sanchez PJ, et al. Early onset neonatal sepsis: the burden of group B streptococcal and *E. coli* disease continues. *Pediatrics*. 2011;127:817–826.
74. Okike IO, Johnson AP, Henderson KL, et al. Incidence, etiology, and outcome of bacterial meningitis in infants aged <90 days in the United kingdom and Republic of Ireland: prospective, enhanced, national population-based surveillance. *Clin Infect Dis*. 2014;59:e150–e157.
75. Lee B, Newland JG, Jhaveri R. Reductions in neonatal listeriosis: "Collateral benefit" of group B streptococcal prophylaxis? *J Infect*. 2016;72:317–323.
76. Schrag SJ, Farley MM, Petit S, et al. Epidemiology of invasive early-onset neonatal sepsis, 2005 to 2014. *Pediatrics*. 2016;138.
77. Greenhow TL, Hung YY, Herz AM, et al. The changing epidemiology of serious bacterial infections in young infants. *Pediatr Infect Dis J*. 2014;33:595–599.
78. Hsieh WS, Tsai LY, Jeng SF, et al. Neonatal listeriosis in Taiwan, 1990-2007. *Int J Infect Dis*. 2009;13:193–195.
79. Lamont RF, Sobel J, Mazaki-Tovi S, et al. Listeriosis in human pregnancy: a systematic review. *J Perinat Med*. 2011;39:227–236.
80. Elinav H, Hershko-Klement A, Solt I, et al. Pregnancy-associated listeriosis: many beliefs, few facts. *Lancet Infect Dis*. 2015;15:1128–1130.
81. Fullerton L, Norrish G, Wedderburn CJ, et al. Nosocomial neonatal *Listeria monocytogenes* transmission by stethoscope. *Pediatr Infect Dis J*. 2015;34:1042–1043.
82. Lazarus C, Leclercq A, Lecuit M, et al. Probable nosocomial transmission of listeriosis in neonates. *J Hosp Infect*. 2013;85:159–160.
83. Silver HM. Listeriosis during pregnancy. *Obstet Gynecol Surv*. 1998;53:737–740.
84. Goulet V, Hebert M, Hedberg C, et al. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clin Infect Dis*. 2012;54:652–660.
85. Fernandez-Sabe N, Cervera C, Lopez-Medrano F, et al. Risk factors, clinical features, and outcomes of listeriosis in solid-organ transplant recipients: a matched case-control study. *Clin Infect Dis*. 2009;49:1153–1159.

86. Guevara RE, Mascola L, Sorvillo F. Risk factors for mortality among patients with nonperinatal listeriosis in Los Angeles County, 1992-2004. *Clin Infect Dis*. 2009;48:1507–1515.
87. Brouwer MC, van de Beek D. Epidemiology of community-acquired bacterial meningitis. *Curr Opin Infect Dis*. 2018;31:78–84.
88. Ku LC, Boggess KA, Cohen-Wolkowiez M. Bacterial meningitis in infants. *Clin Perinatol*. 2015;42:29–45, vii–viii.
89. Thigpen MC, Whitney CG, Messonnier NE, et al. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med*. 2011;364:2016–2025.
90. van Veen KE, Brouwer MC, van der Ende A, et al. Bacterial meningitis in alcoholic patients: a population-based prospective study. *J Infect*. 2017;74:352–357.
91. Mylonakis E, Hohmann EL, Calderwood SB. Central nervous system infection with *Listeria monocytogenes*. 33 years' experience at a general hospital and review of 776 episodes from the literature. *Medicine (Baltimore)*. 1998;77:313–336.
92. Maertens De Noordhout C, Devleesschauwer B, Maertens De Noordhout A, et al. Comorbidities and factors associated with central nervous system infections and death in non-perinatal listeriosis: a clinical case series. *BMC Infect Dis*. 2016;16:256.
93. Skogberg K, Syrjanen J, Jahkola M, et al. Clinical presentation and outcome of listeriosis in patients with and without immunosuppressive therapy. *Clin Infect Dis*. 1992;14:815–821.
94. Brouwer MC, van de Beek D, Heckenberg SG, et al. Community-acquired *Listeria monocytogenes* meningitis in adults. *Clin Infect Dis*. 2006;43:1233–1238.
95. Antolin J, Gutierrez A, Segoviano R, et al. Ciguenza R. Endocarditis due to *Listeria*: description of two cases and review of the literature. *Eur J Intern Med*. 2008;19:295–296.
96. Gauto AR, Cone LA, Woodard DR, et al. Arterial infections due to *Listeria monocytogenes*: report of four cases and review of world literature. *Clin Infect Dis*. 1992;14:23–28.
97. Chavada R, Keighley C, Quadri S, et al. Uncommon manifestations of *Listeria monocytogenes* infection. *BMC Infect Dis*. 2014;14:641.
98. Charlier C, Leclercq A, Cazenave B, et al. *Listeria monocytogenes*-associated joint and bone infections: a study of 43 consecutive cases. *Clin Infect Dis*. 2012;54:240–248.
99. Cardoso C, Cremers I, Oliveira AP. Spontaneous bacterial peritonitis caused by *Listeria monocytogenes*: a case report and literature review. *Ann Hepatol*. 2012;11:955–957.
100. Danion F, Maury MM, Leclercq A, et al. *Listeria monocytogenes* isolation from urine: a series of 15 cases and review. *Clin Microbiol Infect*. 2017;23:583–585.
101. Koufakis T, Chatzopoulou M, Margaritis A, et al. Pneumonia by *Listeria monocytogenes*: a common infection by an uncommon pathogen. *Case Rep Infect Dis*. 2015;2015:627073.
102. Luthe SK, Sato R, Maeda T, et al. *Listeria monocytogenes* meningitis preceded by acute cholangitis. *BMJ Case Rep*. 2017;2017.
103. Legendre C, Hannetel H, Ranc AG, et al. *Listeria monocytogenes* and ocular abscess: an atypical but yet potential association. *Int Ophthalmol*. 2017.
104. Bajor A, Luhr A, Brockmann D, et al. *Listeria monocytogenes* endophthalmitis—case report and review of risk factors and treatment outcomes. *BMC Infect Dis*. 2016;16:332.
105. Hof H. *Listeria* infections of the eye. *Eur J Ophthalmol*. 2017;27:115–121.
106. Clauss HE, Lorber B. Central nervous system infection with *Listeria monocytogenes*. *Curr Infect Dis Rep*. 2008;10:300–306.
107. Le Monnier A, Abachin E, Beretti JL, et al. Diagnosis of *Listeria monocytogenes* meningoencephalitis by real-time PCR for the hly gene. *J Clin Microbiol*. 2011;49:3917–3923.
108. Leber AL, Everhart K, Balada-Llasat JM, et al. Multicenter evaluation of BioFire FilmArray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. *J Clin Microbiol*. 2016;54:2251–2261.
109. Thonnings S, Knudsen JD, Schonheyder HC, et al. Antibiotic treatment and mortality in patients with *Listeria monocytogenes* meningitis or bacteraemia. *Clin Microbiol Infect*. 2016;22:725–730.
110. Hof H, Nichterlein T, Kretschmar M. Management of listeriosis. *Clin Microbiol Rev*. 1997;10:345–357.
111. Safdar A, Armstrong D. Antimicrobial activities against 84 *Listeria monocytogenes* isolates from patients with systemic listeriosis at a comprehensive cancer center (1955-1997). *J Clin Microbiol*. 2003;41:483–485.
112. Seral C, Barcia-Macay M, Mingeot-Leclercq MP, et al. Comparative activity of quinolones (ciprofloxacin, levofloxacin, moxifloxacin and garenoxacin) against extracellular and intracellular infection by *Listeria monocytogenes* and *Staphylococcus aureus* in J774 macrophages. *J Antimicrob Chemother*. 2005;55:511–517.
113. Grumbach NM, Mylonakis E, Wing EJ. Development of listerial meningitis during ciprofloxacin treatment. *Clin Infect Dis*. 1999;29:1340–1341.
114. Baldassarre JS, Ingerman MJ, Nansteel J, et al. Development of *Listeria* meningitis during vancomycin therapy: a case report. *J Infect Dis*. 1991;164:221–222.
115. Hof H. Listeriosis: therapeutic options. *FEMS Immunol Med Microbiol*. 2003;35:203–205.
116. Mitja O, Pigrau C, Ruiz I, et al. Predictors of mortality and impact of aminoglycosides on outcome in listeriosis in a retrospective cohort study. *J Antimicrob Chemother*. 2009;64:416–423.
117. Arslan F, Meynet E, Sunbul M, et al. The clinical features, diagnosis, treatment, and prognosis of neuroinvasive listeriosis: a multinational study. *Eur J Clin Microbiol Infect Dis*. 2015;34:1213–1221.
118. Amaya-Villar R, Garcia-Cabrera E, Sulleiro-Igual E, et al. Three-year multicenter surveillance of community-acquired *Listeria monocytogenes* meningitis in adults. *BMC Infect Dis*. 2010;10:324.
119. Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. *Clin Infect Dis*. 2004;39:1267–1284.
120. van de Beek D, Cabellos C, Dzupova O, et al. ESCMID guideline: diagnosis and treatment of acute bacterial meningitis. *Clin Microbiol Infect*. 2016;22:S37–S62.
121. Olsen SJ, Patrick M, Hunter SB, et al. Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clin Infect Dis*. 2005;40:962–967.
122. American College of Obstetricians and Gynecologists. Management of Pregnant Women With Presumptive Exposure to Listeria monocytogenes; 2018. https://www.acog.org/Clinical-Guidance-and-Publications/Committee-Opinions/Committee-on-Obstetric-Practice/Management-of-Pregnant-Women-With-Presumptive-Exposure-to-Listeria-monocytogenes.
123. Madjunkov M, Chaudhry S, Ito S. Listeriosis during pregnancy. *Arch Gynecol Obstet*. 2017;296:143–152.
124. Gellin BG, Broome CV. Listeriosis. *JAMA*. 1989;261:1313–1320.
125. Brouwer MC, McIntyre P, Prasad K, et al. Corticosteroids for acute bacterial meningitis. *Cochrane Database Syst Rev*. 2015;(9):CD004405.
126. Centers for Disease Control and Prevention (CDC). *FOOD Tool*. Atlanta: CDC; 2018. https://wwwn.cdc.gov/norsdashboard/.

207 *Bacillus anthracis* (Anthrax)

*Gregory J. Martin and Arthur M. Friedlander**

SHORT VIEW SUMMARY

Epidemiology and Microbiology

- Sporadic worldwide, anthrax is most common in Africa, the Middle East, India, Southeast Asia, and Latin America.
- Naturally acquired human cases are usually associated with animal products.
- Spore-forming gram-positive bacillus grows readily in the laboratory.
- When protected from ultraviolet light, spores remain viable for decades or longer.
- Pathogenicity is associated with edema and lethal toxins and a capsule.

Clinical Manifestations and Diagnosis

- *Cutaneous anthrax:* accounts for 95% of naturally acquired cases. After skin inoculation, a pruritic papule forms in 2 to 5 days. Vesicles rupture, leading to formation of a black eschar at the base of a shallow ulcer. An injectional form has more recently been described in people who use injection heroin and is associated with a more aggressive course. Surgical débridement may be required. Gram stain of vesicle fluid, scraping of base of ulcer, or punch biopsy may show gram-positive bacilli and a paucity of polymorphonuclear neutrophils. Culture of material is frequently positive. Direct fluorescent antibody (DFA) test and polymerase chain reaction (PCR) assay also may be used.
- *Inhalational anthrax:* results from handling of animal products such as wool, hides, or bones or after intentional spore release in bioterrorism. This form has the most dangerous presentation, with near 100% mortality without early antibiotics. It is primarily a mediastinal (not an airspace) process. Blood and pleural fluid cultures are positive. Pleural fluid Gram stain may be positive. DFA test or PCR assay may give the most rapid results.
- *Gastrointestinal anthrax:* responsible for approximately 1% of human cases, typically occurring 1 to 5 days after ingestion of contaminated meat. Blood, stool, and ascites all should be obtained for culture, DFA test, and PCR assay. Gram staining of ascitic fluid may reveal gram-positive bacilli.
- *Anthrax meningitis:* secondary seeding of the meninges occurs during bacteremia in fulminant disease. Death occurs within 24 hours in 75% of cases. Cerebrospinal fluid reveals gram-positive bacilli, and cultures are positive. DFA test and PCR assay may provide the most rapid diagnosis.

Therapy

- Rapid initiation of antibiotics for all stages is crucial (see Table 207.2).
- For cutaneous anthrax, ciprofloxacin or doxycycline alone is used.
- Cutaneous anthrax with systemic symptoms, inhalational anthrax, gastrointestinal anthrax, injectional anthrax, and anthrax meningitis should be treated with two bactericidal agents, preferably a quinolone such as ciprofloxacin and a β-lactam such as meropenem, combined with a protein synthesis inhibitor such as linezolid, clindamycin, or chloramphenicol (although not a protein synthesis inhibitor, rifampin may be included as an option). Consider central nervous system penetration of antibiotics for treatment of potential meningitis.
- Anthrax antitoxins such as anthrax immune globulin and monoclonal anti–protective antigen antibodies should be considered in conjunction with antibiotics in severe cases and in some spore exposures.

Prevention

- Current vaccines (anthrax vaccine absorbed [AVA] in the United States and anthrax vaccine precipitated in the United Kingdom) are cell-free supernatants containing protective antigen adsorbed to aluminum hydroxide (AVA) or precipitated with aluminum potassium sulfate (anthrax vaccine precipitated).
- Postexposure vaccination with AVA should be administered at 0, 2, and 4 weeks and administered subcutaneously in conjunction with antibiotics.

Anthrax as an Agent of Bioterrorism

- Anthrax is generally considered the most likely agent for bioterrorism via an aerosol route.
- Gram quantities of stable spores are easy to transport and could cause thousands of cases.
- Early identification of the first cases is difficult owing to the presentation with nonspecific flulike symptoms.
- Nasal swabs are used to identify exposure areas, *not* to determine individual exposures.
- Exposed patients should be given antibiotic prophylaxis with 60 days of ciprofloxacin or doxycycline and anthrax immunization at 0, 2, and 4 weeks.
- Exposed patients should be decontaminated with soap and water. Surfaces may be remediated with a number of different chloride-containing compounds including household bleach.
- Anthrax is *not* transmissible from patients after they have been decontaminated, and isolation is *not* required.

Anthrax has never been a cause of the massive loss of life associated with cholera, plague, or smallpox, but it has played a prominent role in the history of infectious diseases. While much of the industrialized world is focused on anthrax as an agent of bioterrorism, anthrax remains a significant cause of animal deaths as well as more limited numbers of human cases in much of the developing world.

References to a disease that likely was anthrax appear in the Bible, and descriptions of inflamed papules from exposure to tainted wool occur in Virgil's writings.[1] Anthrax was the first disease definitively attributed to a bacterium, which was discovered by Robert Koch in 1877 and was used to first demonstrate Koch's postulates. Louis Pasteur established the concept of attenuating a bacterial pathogen by serial passage of *Bacillus anthracis* and used this approach in 1881 to develop an anthrax vaccine shown to be protective in a field trial in domesticated animals.[2] With the initiation of the factory processing of hides and wool in the industrial age, deaths from inhalational anthrax among 19th-century British and German woolsorters and ragpickers introduced the concept of occupational risks for infectious disease and the need to protect workers from these risks.[3,4] In 1979 an accidental release of anthrax spores from a Soviet military microbiology facility in Sverdlovsk, Russia, was responsible for approximately 70 cases of inhalational anthrax

*The opinions and assertions herein are those of the authors and should not be construed as official or representing the views of the Department of State, Bureau of Medical Services, the Department of Defense, or the US government.

that were originally reported to be gastrointestinal anthrax until details were finally published years later.[5] This outbreak and the revelations that Iraq had produced anthrax spores in 1991[6] raised the possibility of anthrax being used as a weapon. This possibility was realized with the dissemination of anthrax spores from letters sent through the US Postal Service in 2001 that led to 22 cases of human anthrax and 5 deaths and made what had been nearly a forgotten disease in Europe and North America the subject of intense public attention and renewed scientific and medical interest. In the years since the US anthrax attacks there have been significant advances in the understanding of the biology of *B. anthracis,* the pathophysiology of the disease manifestations, and improvements in diagnostic and therapeutic options.

EPIDEMIOLOGY

Anthrax is a worldwide disease of domesticated and wild animals that may secondarily infect humans. Estimates of worldwide cases vary widely, but it is estimated by the World Health Organization (WHO) that there are 2000 to 20,000 human cases per year (Fig. 207.1).[7] Although cases occur worldwide, there is little genetic diversity among isolates. Examination of variable number tandem repeats loci identifies six major clones among two branches. Based on identification of variable number tandem repeats in different geographic areas, it appears that southern Africa has the greatest diversity of strains and is believed to be the geographic origin of *B. anthracis.*[8,9] The actual number of anthrax cases worldwide has been difficult to ascertain owing to poor reporting, but anthrax in animals has been reported from 82 nations. It is significantly more common among grazing herbivores in some areas of the Middle East, Africa, and Latin America than in more developed countries. The enormous areas of savanna and large populations of ungulate herbivores in southern Africa provide an ideal environment for the development of anthrax. In 1923 in South Africa, it was estimated that 30,000 to 60,000 animals died of anthrax.[10] In the last decade, isolates of *Bacillus cereus* strains possessing the virulence plasmids of *B. anthracis* and expressing the anthrax toxins and capsule have been obtained from diverse species of animals in tropical rainforests in sub-Saharan Africa and may threaten the survival of chimpanzees in that area.[11] No human cases with these strains have been reported to date.

The largest human outbreak of anthrax occurred in Zimbabwe during the years 1979–85 with approximately 10,000 reported cases and 182 deaths. These cases, almost all of which were cutaneous, were associated with cattle ranching and lapsed veterinary control practices during the civil war that established the country.[12,13] Anthrax remains enzootic in much of sub-Saharan Africa with continued cases in wildlife and livestock and more cases in humans than in most of the rest of the world combined.[14,15]

In most of Europe and North America, human cases are rare, and animal cases are sporadic and uncommon. A single animal death usually is met with an intense veterinary public health response that mandates proper disposal of carcasses; decontamination of fields; and immunization of surviving, potentially exposed animals. A single human case in a nonagricultural, nonrural setting appropriately raises concern for an act of bioterrorism.

The natural cycle of anthrax infection in humans and animals is illustrated in Fig. 207.2.[16] Many animals that die of anthrax have a characteristic terminal hemorrhage from the nose, mouth, and anus that contains large amounts of anthrax bacilli. Animals are then infected when they graze on fields or grain contaminated with spores or through the bites of flies that have fed on infected carcasses. Seasonal variations in anthrax cases have been noted for decades. Heavy spring rains may serve to concentrate spores into low-lying areas, and if this is followed by a hot, dry period, animals grazing on these areas with high spore burdens may become infected. River beds that dry out after flooding and serve as pasture for animals have been repeatedly implicated.[14] Additionally, in periods of drought, animals grazing on dried grasses close to the soil surface have increased oral abrasions from the dry vegetation. These abrasions provide areas for spore entry and germination and a subsequent increase in animal cases of anthrax.[17] In 2016 anthrax reappeared in the remote Yamal Peninsula in Siberia for the first time in 75 years. As global climate change has melted permafrost, the carcasses of reindeer that had been frozen for decades have thawed and infected reindeer herds and local people with deaths of thousands of reindeer and one human.[18] The Siberian experience demonstrates the resilience of anthrax spores in the right environmental settings. Naturally acquired human cases are usually associated with exposure to infected animals or contaminated animal products. Numerous products have been implicated in transmission to humans including wool, hair, bone and bone meal, meat, horns, and hides. The source may not be readily evident because the animal product may have been processed (e.g., shaving brushes, goat-skin drums, wool-based tapestries, and bone meal–based fertilizers).[19,20,21,22] In 2013 an inhalational anthrax case occurred in an American who had traveled through four US states with endemic anthrax. Despite an extensive investigation, it appears his only risk factor was driving through herds of bison and burros a few days before developing symptoms.[23] Transmission from flies has also been documented; biting flies may carry spores or vegetative forms from a carcass to another animal or human, and even nonbiting

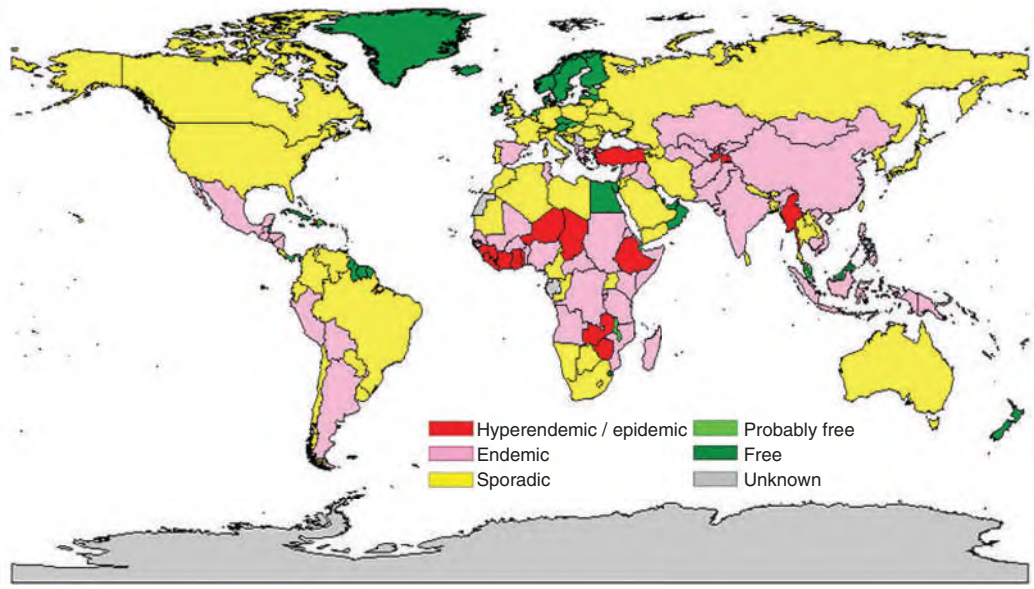


FIG. 207.1 World distribution of anthrax cases as compiled by the World Health Organization. *(From Louisiana State University School of Veterinary Medicine. Welcome to the World Anthrax Data Site.* http://www.vetmed.lsu.edu/whocc/mp_world.htm. *Accessed March 26, 2014.)*

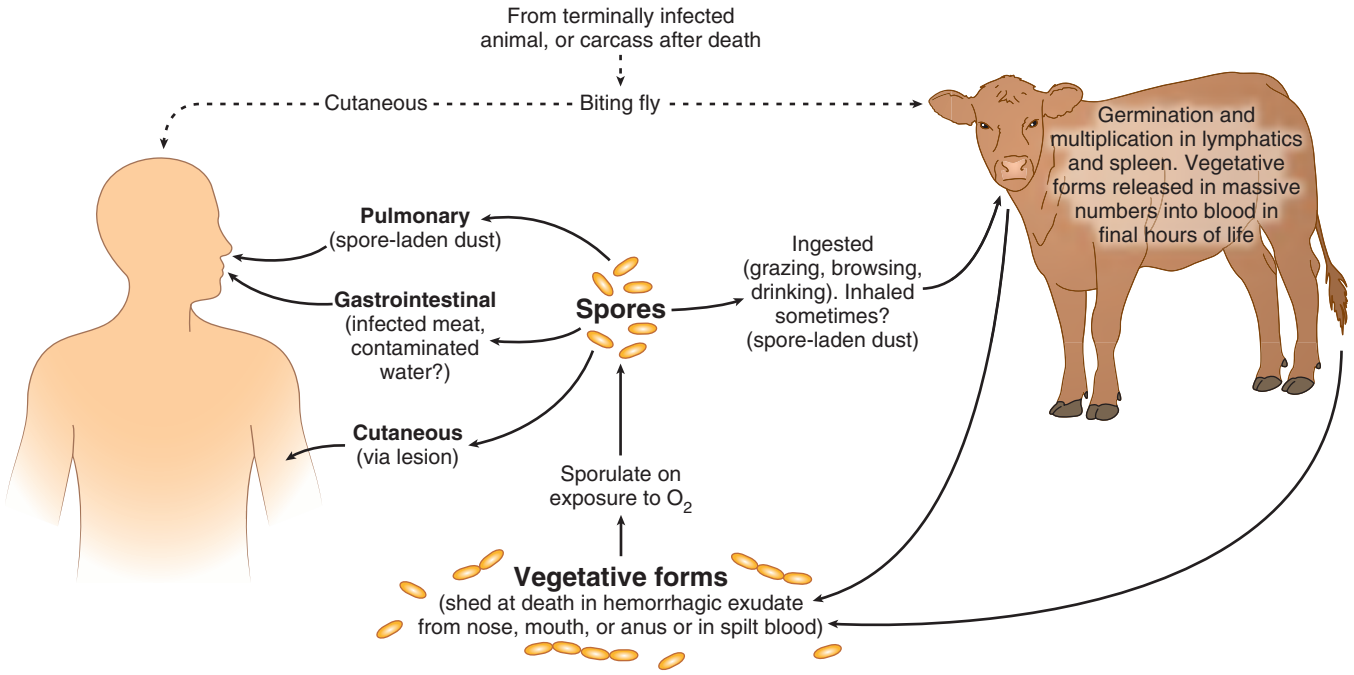


FIG. 207.2 Cycle of infection in anthrax. The spore is central to the cycle, although vegetative forms may also play a role in establishing infection when, for example, humans or carnivores eat meat from an animal that died of anthrax or when biting flies transmit the disease. *(From World Health Organization.* Anthrax in Humans and Animals. *4th ed. Geneva: WHO Press; 2008.)*

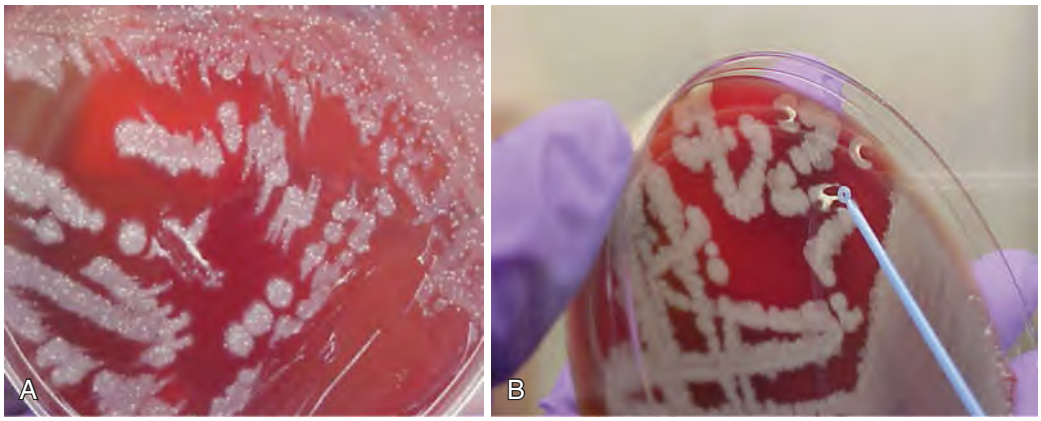


FIG. 207.3 Appearance of *Bacillus anthracis* colonies. (A) Colonies of *B. anthracis* on sheep blood agar demonstrating white-gray colonies and "comet trail" or "Medusa's head" outgrowths from colony margins. (B) "Whipped egg white" appearance of tenacious *B. anthracis* colonies while being removed from sheep blood agar. *(Courtesy Robert Paolucci, National Naval Medical Center, Bethesda, MD.)*

flies have been shown to carry *B. anthracis* in feces or vomit that they deposit onto vegetation. Birds such as vultures shed anthrax spores in their feces for up to 2 weeks after they ingest infected meat.[24] In Europe, people who used injection drugs developed anthrax infections from injecting heroin contaminated with spores possibly acquired from the goat skin containers used in the transport of the drug from Turkey.[25-23]

Spores are the usual infecting form of anthrax. However, ingestion of either spore or vegetative forms of *B. anthracis* in contaminated meat may lead to gastrointestinal infection.

The world distribution of anthrax cases in humans and animals is tracked via a geographic information system and remote sensing by WHO as part of the World Anthrax Data Site. This includes an updated nation-by-nation breakdown of cases by species and year.[7]

MICROBIOLOGY

B. anthracis, the causative agent of anthrax, is a large (1–1.5 µm × 3–8 µm), gram-positive bacillus with rapid, nonhemolytic growth on blood agar that readily forms spores in the presence of oxygen. Colonies have a characteristic "Medusa's head appearance," sometimes also referred to as a "comet tail," appearing slightly curled at the periphery (Fig. 207.3A). The white or gray-white colonies are tenacious when attempts are made to remove them from agar, and this is often described as "a whipped egg white appearance" when a loop is passed through a colony (Fig. 207.3B). In culture the bacilli may form long chains with prominent central or paracentral oval spores that do not cause swelling of the bacilli (Fig. 207.4). In infected tissue, bacteria occur singly or in short chains of two to three bacilli without spores. In the presence of carbon dioxide in the laboratory, or of bicarbonate in tissue, *B. anthracis* forms a prominent poly-D-γ-glutamic acid capsule important in the inhibition of phagocytosis of the vegetative bacilli. Catalase positivity and nonmotility of organisms are further characteristics that differentiate *B. anthracis* from other *Bacillus* spp. These basic identification techniques can typically be performed in nearly all microbiology laboratories, but definitive identification of *B. anthracis* requires further demonstration of lysis by γ phage, detection of the capsule

by fluorescent antibody, and identification of toxin genes by polymerase chain reaction (PCR) assay, usually best performed at a reference laboratory.[26,27]

In contrast to growth during in vitro cultivation, *B. anthracis* sporulation does not occur in viable tissues until they are exposed to atmospheric levels of oxygen, typically after an infected animal has died and the carcass is opened. The spores, although sensitive to prolonged ultraviolet radiation, are extremely hardy and may survive in certain soil conditions for decades. In the interior of buildings, typically shielded from ultraviolet light, spores may also remain viable for years. Although anthrax spores have demonstrated viability in soil and carcasses for decades, and even longer in bones from an archeological site,[28] in most environments where the organism must compete with other soil-dwelling bacteria they typically survive only for months and rarely more than 4 years.

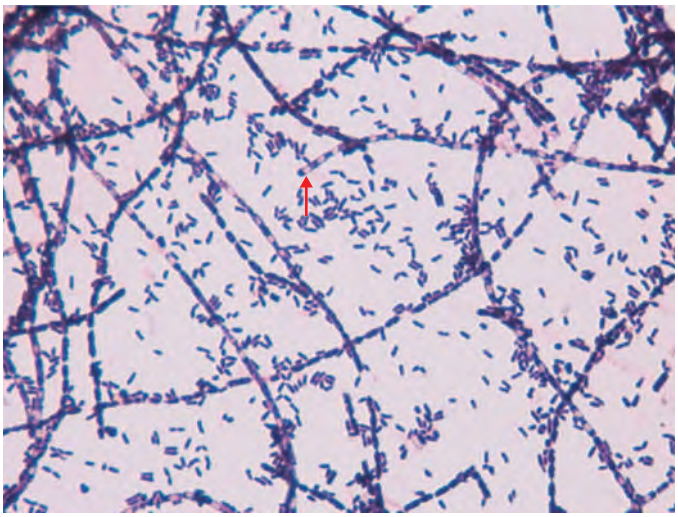


FIG. 207.4 Gram stain of *Bacillus anthracis* demonstrating long chains of bacilli that form when grown in culture. The prominent central or paracentral spores do not stain with Gram staining and appear as clear areas in many of the bacilli in chains (example shown at *red arrow*). *(Courtesy Robert Paolucci, National Naval Medical Center, Bethesda, MD.)*

Spores may also remain dormant, but viable, in living animals for a period of at least 100 days, as demonstrated by primate studies in which viable organisms were recovered at necropsy from the lungs of apparently healthy animals,[29–31] a finding that has important therapeutic implications, as discussed later.

The two major virulence factors are the antiphagocytic poly-D-γ-glutamic acid capsule, encoded on the pX02 plasmid, and two exotoxins, encoded on a separate plasmid, pX01. The anthrax toxins have been intensively studied, and components of the toxins have important use in vaccines, in diagnostics, and as targets for new adjunctive therapeutics. A schematic representation of the main anthrax toxins in shown in Fig. 207.5.[32]

The pX01 plasmid encodes three toxin components, known as protective antigen (PA), edema factor (EF), and lethal factor (LF), each of which individually is biologically inactive. Studies in the 1950s and 1960s established that edema toxin, composed of PA combined with EF, produced local skin edema and that lethal toxin, composed of the same PA combined with LF, was highly lethal for experimental animals. The combination of all three components was the most lethal and produced many characteristics of an actual anthrax infection.[33]

PA, which was originally identified as an antigen that induced protective immunity in experimental animal models, attaches to the anthrax toxin receptors—tumor endothelial marker 8 (TEM8) and capillary morphogenesis protein 2 (CMG2)—on the cell surface and is cleaved by a cell surface protease into PA_{63}, which forms a heptamer to which up to three EF and/or LF molecules may attach.[34] CMG2 appears to be the major toxin receptor in the mouse model in which neutrophils contribute to resistance to infection.[35] PA may also be cleaved by a serum protease with formation of toxins in the circulation. When the PA heptamer complexes with EF, it forms edema toxin, and with LF it forms lethal toxin. The toxins are then taken into the cytosol, where they mediate cellular damage. LF is a calmodulin-dependent zinc metalloprotease that cleaves and inactivates multiple mitogen-activated protein kinases and interferes with signal transduction, whereas EF is an adenylate cyclase that increases intracellular cyclic adenosine monophosphate concentrations and interferes with cell function. The toxins have been shown in vitro to impair cell functions associated with innate immunity including neutrophil chemotaxis; phagocytosis; superoxide production; and macrophage, T and B lymphocyte, and dendritic cell function[36] and likely affect many other cells possessing toxin receptors including endothelial cells resulting in increased vascular

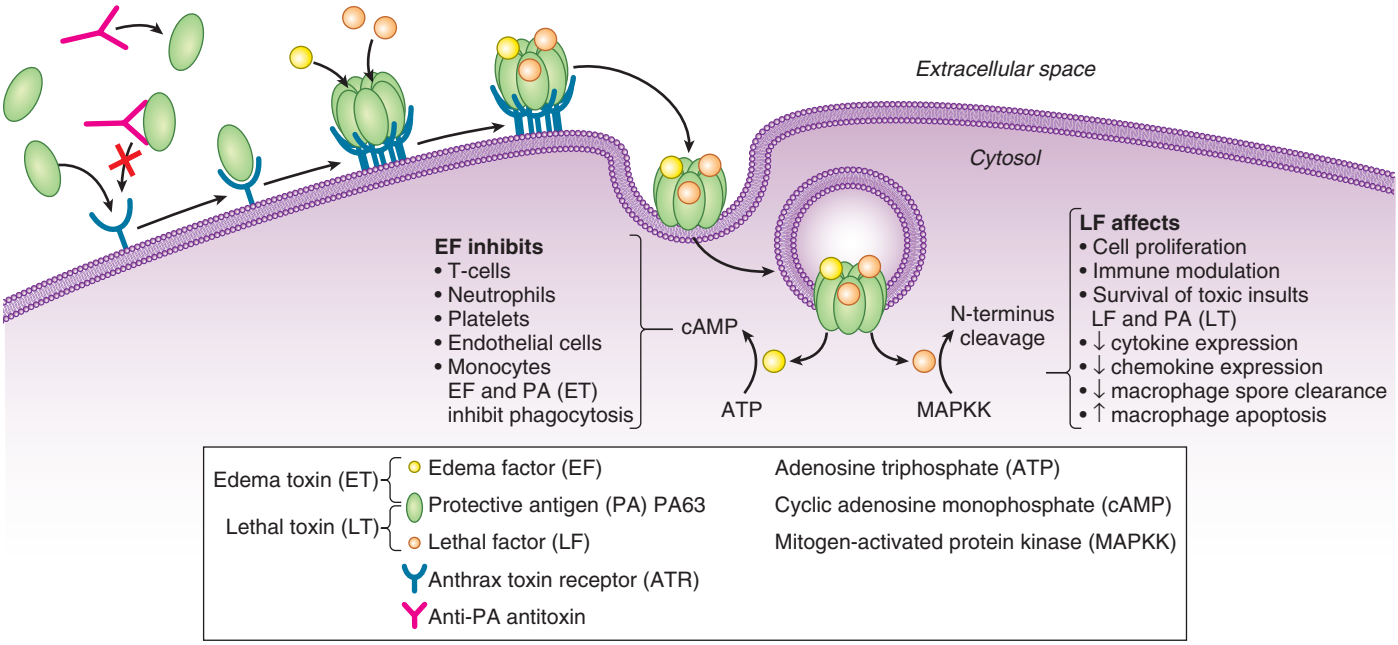


FIG. 207.5 Formation and activity of main anthrax toxins. *(From Bower W, Hendricks K, Pillai S, et al. Clinical framework and medical countermeasure use during an anthrax mass-casualty incident.* MMWR Morb Mortal Wkly Rep. *2015;64:1–23.)*

permeability[37] and cells of the cardiovascular system and liver.[35] Studies with isolated toxins suggest they both cause hypotension in experimental animals and are additive, but shock has not been a prominent finding in patients presenting with inhalational anthrax. Edema toxin was so named because of its ability to cause edema in experimental animals, and lethal toxin has also been shown to increase vascular permeability. Lethal toxin demonstrates effects on the intestinal epithelium that may allow for secondary infections with enteric pathogens.[38] However, there are discrepancies between effects of toxins observed in cells in vitro and in vivo and findings observed in experimental infections. The cell targets and exact role and mechanisms of toxin-induced host dysfunction during infection remain under investigation, and numerous other bacterial factors contribute to virulence, although they are of less importance than the capsule and toxins.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

The clinical manifestations of anthrax in animals and humans have been well described since the 1800s, when cases were relatively common in many areas of the world. The three primary forms of anthrax are dependent on the route of exposure: cutaneous, gastrointestinal, and inhalational. In the past decade an additional form of cutaneous anthrax has been described among people who inject heroin. Heroin contaminated by *B. anthracis* spores may cause severe systemic infection similar to that seen in advanced inhalational anthrax.[39,40] Bacteremia secondary to any of the primary forms of anthrax may lead to seeding of any site including the central nervous system (CNS), with the resulting hemorrhagic meningoencephalitis being nearly always fatal.

Online resources for clinicians considering a diagnosis of anthrax are readily available. Frequently updated and extensively referenced anthrax websites for both naturally occurring and bioterrorism-associated disease are provided by the Centers for Disease Control and Prevention (CDC) (www.cdc.gov/anthrax/), the European Centre for Disease Prevention and Control (https://ecdc.europa.eu/en/anthrax), and the Center for Infectious Disease Research and Policy (http://www.cidrap.umn.edu/infectious-disease-topics/anthrax). Additional resources are provided by WHO at www.who.int/csr/disease/Anthrax/resources/en/. The WHO site features a comprehensive handbook of WHO guidelines for anthrax in animals and humans that includes information on handling of carcasses, disinfection, and control of infections.[16] Laboratory guidelines[26] and guidelines for shipping and handling of clinical specimens[41] are continually revised by the American Society for Microbiology and are available at https://www.asm.org/index.php/guidelines/sentinel-guidelines.

Cutaneous Anthrax

Naturally occurring anthrax infections in humans cause cutaneous disease in more than 95% of cases. Series of cutaneous anthrax cases from the 19th century and early 20th century that were untreated demonstrated 16% to 39% mortality. As anthrax immune serum was used for treatment in cutaneous case series in the years 1903–41, mortality decreased to 0% to 28%, although no controlled studies were reported. With penicillin treatment available, mortality rates have generally decreased to less than 5%.[42] In a series from 1955, Gold[43] carefully reported the findings of 117 cases of anthrax he observed near a Philadelphia goat-hair mill over 20 years. All but one case was cutaneous anthrax, and the only fatalities were the single pulmonary case and one cutaneous case. The Chinese Center for Disease Control and Prevention reported 120,111 human cases of anthrax for the years 1955–2014 with 4341 fatalities (3.6%). Of 258 confirmed Chinese anthrax cases for the years 2005–14, 98% were cutaneous anthrax, and only 2% of all anthrax cases were fatal.[44]

As described earlier, a multitude of contaminated animal sources have been implicated in naturally acquired cutaneous anthrax in humans. After the introduction of anthrax spores into the skin, often with just trivial trauma, there is an incubation period of 1 to 19 days (more commonly 2–5 days), leading to the development of a small, pruritic papule at the inoculation site.[45] Most lesions are on exposed areas of the head, neck, and extremities. Owing to the associated pruritus, patients (and clinicians) often attribute these painless lesions to an insect or spider bite and ignore them.

A day or two after the formation of the papule, vesicles form around the lesion and may become 1 to 2 cm in diameter. The vesicles contain a clear to serosanguineous fluid, and Gram stain reveals numerous bacilli but a paucity of leukocytes. Culture of vesicular fluid will readily yield *B. anthracis* in most cases in which antibiotics have not been administered. There is no purulence, and the lesions remain painless unless secondarily infected. The vesicles are thin roofed and easily rupture, leading to formation of a dark brown eschar that turns black at the base of a shallow ulcer. The ulcer is typically surrounded by an area of induration, and in some cases nonpitting edema may be marked (Fig. 207.6).[46] Most other organisms causing skin infections are not typically associated with extensive induration around a skin lesion or with frank edema, so these findings may be the first clue to the diagnosis of anthrax. As the ulcer matures, its base becomes characteristically black and is the source of the name anthrax, which derives from the Greek word *anthrakis,* meaning "coal."

In uncomplicated cases (i.e., without secondary spread), lesions slowly heal over a period of 1 to 3 weeks, and the eschar loosens and falls off, typically without leaving a scar. Antibiotics do not affect the evolution of the skin lesions. In most cases, patients report associated headache, malaise, and low-grade fever even if the infection does not progress to bacteremia.

Multiple lesions may occur in some cases, probably representing multiple inoculation sites, but at other times small satellite lesions may appear around an initial isolated lesion. Serious cutaneous disease may be marked by extensive edema that involves an entire extremity or the trunk from neck to groin. This has been described as "malignant edema" and may be associated with inflammation of the overlying skin with pain, signs of toxemia, and subsequent secondary seeding of other sites as bacteremia develops; cutaneous anthrax with severe edema has been more commonly seen in injectional anthrax (see later discussion).

Untreated cutaneous disease in humans has been associated with a fatality rate of 10% to 20%, whereas treated cutaneous disease (before the onset of secondary bacteremic spread) is rarely fatal. Repeat infections are rarely reported and tend to be milder, implying some degree of acquired immunity.[43,45]

Clinical characteristics that should place cutaneous anthrax high in the differential diagnosis include a painless lesion (during initial stages), the presence of edema out of proportion to the size of the lesion, and a Gram stain of vesicular fluid or ulcer swab with gram-positive rods but rare white blood cells.

Differential Diagnosis of Cutaneous Anthrax

The differential diagnosis of the unusual skin lesions associated with cutaneous anthrax includes some diagnoses uncommonly encountered by most clinicians. Brown recluse spider bites, which may also cause a black eschar and some associated edema, may be confused with cutaneous anthrax lesions. The key difference is the significant pain associated with a recluse spider bite and the absence of pain in anthrax lesions (although there may be tender adenopathy in association with an anthrax skin lesion). The differential diagnosis of cutaneous anthrax also includes tularemia, scrub typhus, rat-bite fever, blastomycosis, cutaneous fungus acquired from animals, and mycobacterial infection with *Mycobacterium marinum.*

Laboratory Diagnosis of Cutaneous Anthrax

Diagnostic procedures for cutaneous anthrax should preferably be performed before initiation of antibiotics because vesicular fluid and biopsy material are quickly rendered noninfectious after initiation of antibiotics. Appropriate samples for Gram stain and culture include vesicle fluid, either in a syringe or on a swab; a specimen from swabbing the edge of the base of an eschar; and material from a full-thickness punch biopsy of the edge of a vesicle and/or the center of an eschar (Table 207.1). A *Bacillus* species in a culture specimen can be confirmed as *B. anthracis* by demonstration of bacterial lysis in the presence of the γ phage, detection of the capsule by direct fluorescent antibody (DFA), and identification of toxin genes by PCR assay as described later (all generally available in reference laboratories).[26]

Since the anthrax attacks in the United States in 2001, a two-component DFA assay that uses fluorescein-labeled monoclonal

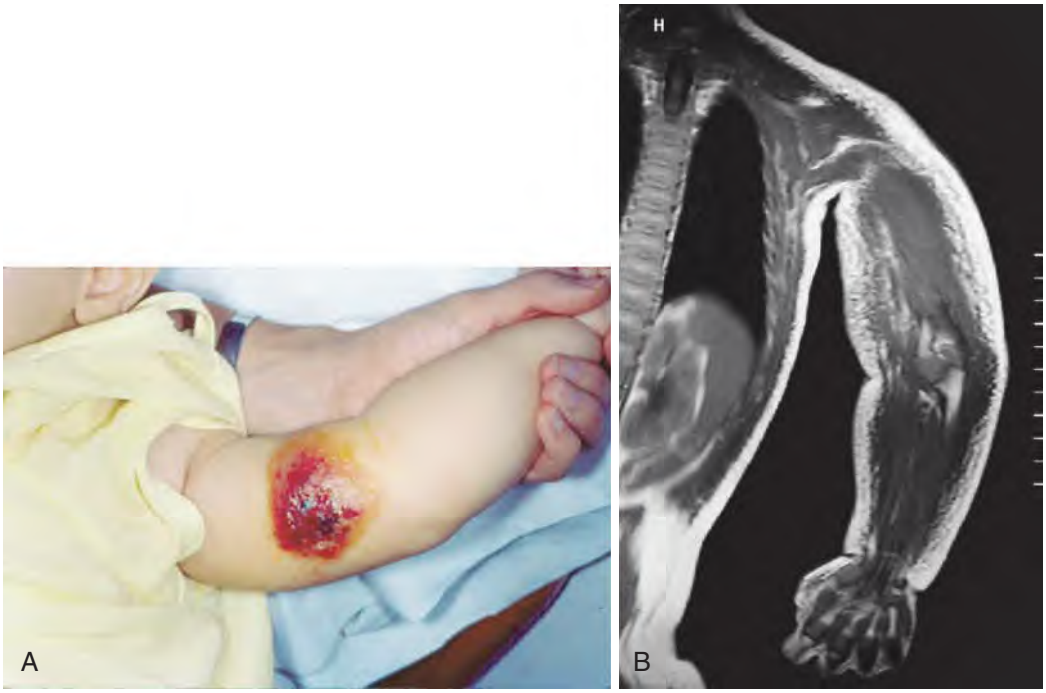


FIG. 207.6 Clinical and magnetic resonance imaging appearance of cutaneous anthrax. (A) Cutaneous anthrax with extensive nontender swelling and erythema in a 7-month-old child in New York in 2001. (B) Magnetic resonance imaging demonstrates extensive subcutaneous edema from shoulder to hand. *(From Roche KJ, Chang MW, Lazarus H. Cutaneous anthrax infection: images in clinical medicine.* N Engl J Med. *2001;345:1611. Copyright © 2001 Massachusetts Medical Society. All rights reserved.)*

antibodies (MAbs) specific to the *B. anthracis* cell wall and capsule has been developed.[47,48] Rapid PCR assays are also now available.[49,50] Testing may be obtained through the CDC Laboratory Response Network (local Sentinel laboratories, city or state Reference laboratories, or CDC and military National laboratories) and some hazardous material teams. DFA and real-time PCR can be used for rapid and definitive identification of culture isolates and for presumptive identification of *B. anthracis* directly from clinical specimens and, in some cases, environmental samples. Caution must be used in interpreting these results because false-positive and false-negative findings may occur. In even the most experienced laboratories, cross-contamination is always a risk with PCR, and false-positive results can lead to considerable confusion.[51]

Because blood cultures are frequently positive in cases that have progressed to sepsis, consideration should be given to obtaining blood cultures early in the evaluation, especially if there are any systemic symptoms. Automated blood culture systems commonly used in hospitals readily support growth of *B. anthracis*.

Culture of *B. anthracis* remains the gold standard for diagnosis of anthrax infections. Table 207.1 outlines diagnostic specimen preparation, handling, and testing. Despite development of molecular diagnostics for anthrax, there is still a role for serology. Three of the cutaneous cases in 2001 had no culture or PCR evidence of disease, but acute and convalescent serology demonstrated an anti-PA antibody response that confirmed the diagnosis. Acute and convalescent serum samples should be obtained for serology at 0 to 7 days of illness and at 14 to 28 days. A rapid enzyme-linked immunosorbent assay (ELISA) that measures total antibody to PA has been approved. A number of anthrax-PA ELISA kits have been approved by the US Food and Drug Administration (FDA) and can be used on serum to diagnose all types of anthrax or to demonstrate seroconversion after immunization. Retrospectively, anthrax PA antibody was detected by ELISA in 100% of both cutaneous and inhalation cases from 2001. However, ELISA is not positive early in disease; PA antibody is not detected until approximately 1 week after symptoms begin.[27,52]

Anthrax diagnostics for both environmental and clinical samples continue to be developed. A number of lateral flow devices (handheld assays) designed for environmental samples are available. Although easy to perform and more rapid than other diagnostics, they are typically not as sensitive or specific as more traditional methods and should not be used on clinical specimens.

Injectional Anthrax

Injectional anthrax is an uncommon form of cutaneous anthrax that has been described in people who use injection drugs who introduced contaminated heroin either into the skin or intravenously. Both *Clostridium* and *Bacillus* spp. (usually *B. cereus*) are spore-forming organisms that have been previously associated with infections in people who use injection drugs. In 2009 a few sporadic injectional anthrax cases occurred in Europe; in 2009–10 there were 119 cases among people who injected heroin in the United Kingdom, mainly in Scotland. Injectional anthrax is difficult to diagnose because skin infections are common among people who use injection drugs, but the clinical presentations of injectional anthrax cases were atypical. Skin lesions were not typical cutaneous anthrax lesions; rather, patients presented with advanced localized soft tissue infections accompanied by disproportionate edema, often with less pain than is typically associated with other serious soft tissue infections such as necrotizing fasciitis. Fever was not a prominent feature. Some patients had no localized injection-related lesions but presented with features of systemic anthrax infection; deteriorated rapidly; and died with meningitis, sepsis, and multiorgan failure. Patients were treated with conventional antibiotics, and some required extensive surgical débridement for necrosis associated with deep infections and subsequent reconstructive surgery; this is in contrast to typical cutaneous anthrax, for which surgical débridement is not recommended. Fourteen of the patients also received intravenous (IV) therapy with anthrax immune globulin (AIG).[25,53]

Inhalational or Pulmonary Anthrax

Even a single case of inhalational anthrax should raise the possibility of a deliberate spread of spores because naturally occurring inhalational disease is currently extraordinarily rare. Inhalational anthrax is an exceptionally dangerous type of *B. anthracis* infection that in the preantibiotic era was nearly uniformly fatal. In a review of 82 reported cases of inhalational anthrax in the years 1900–2005, there was an

TABLE 207.1 Collection and Transport of Laboratory Specimens for Diagnosis of Anthrax

TYPE OF ILLNESS	SPECIMEN COLLECTION AND TRANSPORT	COMMENTS
Cutaneous anthrax	*All stages:* Collect two swabs, one for Gram stain and culture and one for PCR assay.	*Swabs:* Moisten with sterile saline or water; transport in sterile tube at 2°C–8°C.
	Vesicular stage: Perform Gram stain, culture, and PCR assay of fluids from unroofed vesicle (soak two dry sterile swabs in vesicular fluid). *Note:* Gram stain is most sensitive during vesicular stage.	Transport swabs for PCR assay only at –70°C. Do not use transport medium.
	Eschar stage: Perform Gram stain, culture, and PCR assay of ulcer base or edge of eschar without removing it.	*Tissue, fresh:* ≥5 mm^3; store and transport at 2°C–8°C (≤2 h) or frozen at –70°C (>2 h).
	Ulcer (no vesicle or eschar present): Swab base of ulcer with premoistened sterile saline.	*Tissue, preserved in 10% buffered formalin:* 1.0 cm^3; store and transport at room temperature.
	A punch biopsy for IHC testing and a second biopsy for culture, Gram stain, PCR assay, and frozen tissue IHC if patient has not received antibiotics should be obtained on all patients with suspected cutaneous anthrax. Include skin adjacent to papule or vesicle. If vesicles and eschars are both present, separate biopsy specimens should be obtained.	Obtain biopsy specimen of lesions for histopathology, preserved in 10% buffered formalin: 0.3 mm diameter; store and transport at room temperature.
	Serum: Collect acute serum within first 7 days of symptom onset and convalescent serum 14–35 days after symptom onset.	Freeze serum after separation at –20°C or colder, ship on dry ice. Ship part of sample (>1.0 mL) and retain part in case of shipping problems.
	Collect blood for culture and PCR assay and serum for LF detection with evidence of systemic involvement.	Obtain blood for culture per local protocol. Collect blood for PCR assay in EDTA (purple top) tube. Ship at room temperature (≤2 h transport) or 2°C–8°C (>2 h transport). Assay for serum LF toxin and presence of capsule available at CDC.
Inhalational anthrax	If sputum is being produced, collect sputum specimen for Gram stain and culture (*note*: inhalational anthrax does not usually result in sputum production).	*Sputum:* Transport at room temperature in sterile, screw-capped container (<1 h transport time) or at 2°C–8°C (>1 h transport time).
	Obtain blood for smear, culture, and PCR assay and serum for LF detection.	*Blood cultures:* Obtain appropriate blood volume, number, and timing of sets per laboratory protocol; transport at room temperature.
	If a pleural effusion is present, collect a specimen for culture, Gram stain, PCR assay, and LF detection.	*Blood for PCR assay:* 10 mL in EDTA (for pediatric patients collect volumes allowable). Transport directly to laboratory at room temperature (2°C–8°C if transport ≥2 h).
	Collect CSF if meningeal signs are present or meningitis is suspected for culture, Gram stain, PCR assay, and LF detection.	*Pleural fluid:* Collect >1 mL in sterile container. Store and transport at 2°C–8°C.
	Serum: Collect acute serum within first 7 days of symptom onset and convalescent serum 14–35 days after symptom onset.	*CSF:* Transport directly to laboratory at room temperature, or 2°C–8°C if transport ≥2 h.
	Biopsy material: Bronchial or pleural biopsy material can be evaluated if available.	Transport serum or citrated plasma (separated and removed from clot) at 2°C–8°C (transport <2 h) or freeze at –20°C or colder (transport ≥2 h); ship on dry ice. Ship part of sample (>1.0 mL) and retain part in case of shipping problems.
		Preserve biopsy specimens in 10% buffered formalin, and transport at room temperature.
Gastrointestinal anthrax	Obtain stool specimen for culture (>5 g).	*Stool:* Transport in sterile container unpreserved (≤1 hr transport time) or at 2°–8°C in Cary-Blair medium or equivalent (>1 hr transport time); specimen >5.0 g.
	Obtain rectal swab from patients unable to produce stool (insert swab 1 inch beyond anal sphincter).	*Blood:* Transport at room temperature.
	Obtain blood for smear and culture (and possibly PCR testing and LF detection). Blood cultures most likely to yield *Bacillus anthracis* if taken 2–8 days postexposure and before administration of antibiotics.	
	If ascites is present, obtain a specimen for Gram stain and culture (and possibly PCR testing and LF detection).	
Anthrax meningitis	Obtain CSF specimen for Gram stain, culture, PCR assay, and LF detection.	See comments above for collection and transport of blood and CSF for Gram stain, culture, PCR assay, and LF detection.
	Obtain blood for Gram stain, culture, and PCR assay, and serum for LF detection.	

CDC, Centers for Disease Control and Prevention; *CSF,* cerebrospinal fluid; *EDTA,* ethylenediaminetetraacetic acid; *IHC,* immunohistochemistry; *LF,* lethal factor; *PCR,* polymerase chain reaction.
Modified from Center for Infectious Disease Research and Policy. Anthrax: Clinical Laboratory Testing. http://www.cidrap.umn.edu/infectious-disease-topics/anthrax#overview&1-5.

overall 92% mortality rate despite treatment with anthrax antiserum or antibiotics or both in the majority of cases.[54] During the 2001 anthrax attacks in the United States, 5 of the 11 (45%) patients with inhalational anthrax died despite aggressive intensive care unit (ICU) management and appropriate antibiotics. Early diagnosis, initiation of antibiotics, and aggressive management of inhalational anthrax are crucial to survival.

During the 19th century, inhalational anthrax (woolsorter's disease) occurred among factory workers handling hair, wool, or hides contaminated with anthrax spores, with studies demonstrating that as many as 50% of samples of raw materials were contaminated with spores. In the Bradford district of England, 23 cases of inhalational anthrax were reported during the year 1880. Much of our experience with naturally acquired inhalational anthrax was gained in the preantibiotic era.[55,56] Studies in the 1950s revealed that during an 8-hour period mill workers inhaled hundreds of spores smaller than 5 µm, and some had positive nasal or pharyngeal cultures, and yet inhalational anthrax remained extraordinarily uncommon.[56] In a serologic study of unvaccinated mill employees, nearly 15% had antibodies to anthrax.[57] It is evident that there is some threshold number of spores that can be destroyed through the innate immune response even in the absence of prior immunization.[58,59] As safeguards were built into the process so that wool was decontaminated before handling by workers and ventilation was improved in factories, the number of annual cases in developed countries in the second half of the 19th century decreased significantly; with the addition of vaccines and respirators in the 1950s and 1960s, cases dropped nearly to zero.

In 2005 Lucey[60] proposed a modified three-level staging system for inhalational anthrax characterized by an early prodromal stage leading to the intermediate progressive stage followed by the late fulminant

stage that has generally become accepted as reflecting the course of both terrorist-associated and recent naturally occurring inhalational anthrax and is used here. As spores are inhaled, those larger than 5 μm are captured in the upper airways and transported out of the airways via the mucociliary elevator to the mouth. Spores in particles smaller than 5 μm may reach the terminal bronchioles and alveoli, where they are quickly phagocytized by alveolar phagocytic cells and transported to draining lymph nodes and then to mediastinal lymph nodes. More recent studies have suggested that spores may be transported to lymphatics through alveolar epithelial cells more commonly than phagocytes.[61] This early prodromal stage is a clinically silent incubation period and is the presymptomatic stage of inhalational anthrax occurring 1 to 6 days after initial exposure. Although it has been extremely rare to see inhalational cases develop more than 1 week after natural exposure, significant controversy exists about potential incubation periods of 60 days or longer after very-low-dose exposure.[62,63]

The first symptoms occur in the early prodromal stage with a flulike illness characterized by low-grade fever, malaise, fatigue, and myalgias usually without upper respiratory tract symptoms. Headache may be prominent, fatigue may be profound, and blurred vision and photophobia occur in some cases. Dry cough and mild precordial discomfort are also seen in some patients. Patients may experience a biphasic illness during which they feel somewhat improved after the 2 to 3 days of the prodromal illness, whereas others progress directly to the intermediate progressive stage associated with high fever, declining pulmonary status, respiratory distress, dyspnea, marked diaphoresis, pleuritic chest pain, and confusion or syncope. Blood cultures are typically positive in this stage, and mediastinal widening and pleural effusions are noted radiographically. Diagnosis during this stage and treatment with appropriate multiple antibiotics as well as antitoxin therapies (AIG and/or MAbs) coupled with intensive supportive care are associated with survival in most cases.

With or without therapy patients may progress to the late fulminant stage (often referred to in older literature as the fulminant acute phase). These patients have some combination of respiratory failure requiring intubation, sepsis, meningitis, and multiorgan failure associated with overwhelming bacteremia/toxemia. Death frequently occurs within 24 hours. In addition to aggressive antibiotics and intensive care management, these patients should be considered for treatment with antitoxin therapy such as AIG and anthrax MAbs.[59–64]

Inhalational anthrax is a mediastinal process and not a primary airspace disease. Although the majority of inhaled spores are believed to germinate into vegetative organisms while being carried to (or after arrival in) the mediastinal lymph nodes, studies in nonhuman primates have demonstrated that some spores remain dormant for weeks to months.[31] As the vegetative bacilli destroy and burst out of the cell that transported them across the alveoli, they become encapsulated and are released into the systemic circulation, leading to seeding of multiple organs including the meninges. Vegetative bacteria reach high levels in the blood and may be visible on staining of the buffy coat. Levels of the lethal toxin may become high enough terminally that a bacteria-free serum sample may contain enough toxin to kill another animal. The initial signs and symptoms of inhalational anthrax are not very specific, and discriminating between early inhalational anthrax and influenza can be quite difficult, although the characteristic upper respiratory tract symptoms found with influenza are usually absent in anthrax.[54,65]

Chest radiography, or more commonly computed tomography (CT), reveals a widened mediastinum and often bilateral pleural effusions. The progression of inhalational anthrax with chest radiographs and CT from a 2001 bioterrorism case is seen in Fig. 207.7.[66] Before the bioterrorist-associated anthrax cases in 2001, it was generally accepted that pulmonary infiltrates or consolidation were not typically prominent in inhalational anthrax (because it is not a primary parenchymal lung disease), but 7 of 10 inhalational cases in the 2001 attacks were noted to have pulmonary infiltrates. However, it was found that areas of pulmonary infiltrate on chest radiography actually corresponded to pulmonary edema and hyaline membrane formation at necropsy, not pneumonia with bacterial infiltration of the alveoli.[67,68] Pleural effusions are seen in most cases and are typically serosanguineous or hemorrhagic. They may rapidly reaccumulate after thoracentesis, requiring drainage with tube thoracostomy. Adequate pleural fluid drainage is important to achieve because it was associated with a significant survival advantage in the meta-analysis of 82 inhalational cases.[54]

Diagnosis of Inhalational Anthrax

Table 207.1 outlines guidelines for diagnostic specimen preparation, handling, and testing, which are generally identical to guidelines described for cutaneous anthrax earlier. Although inhalational anthrax is typically not associated with a cough productive of sputum, if sputum is produced, it should be sent for Gram stain, culture, and PCR analysis. Pleural fluid is more frequently present and should be sent for diagnostic testing because it is much more likely to yield bacilli on staining, culture, or PCR assay. Much more commonly than in cutaneous anthrax, the diagnosis of inhalational anthrax is made by finding positive blood cultures, and these should be obtained before any antibiotics are administered. Especially in patients who have received antibiotics, blood samples should be sent for PCR assay and antigen detection. Buffy coat smears can also be examined for the presence of bacilli, an ominous sign that the patient has entered the late fulminant stage of anthrax. Immunohistologic studies of tissue specimens for the presence of bacillus cell wall and capsule antigens may be of particular value in treated patients because results may be positive when culture, Gram stain, and PCR are negative.[52]

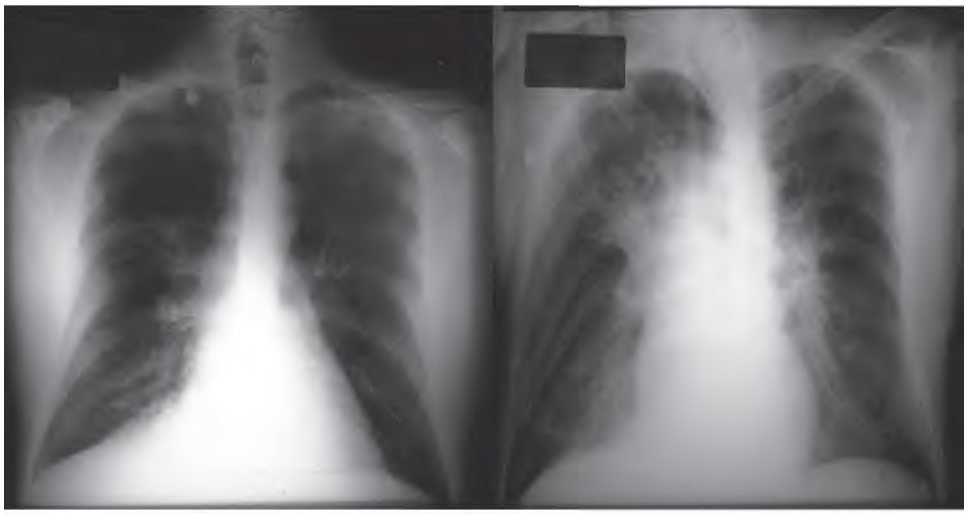
Gastrointestinal Anthrax

Oropharyngeal or intestinal infections with *B. anthracis* are indicative of gastrointestinal anthrax. This form of anthrax is quite common in the grazing herbivores that are the usual hosts for anthrax infections but is uncommon in humans, responsible for only approximately 1% of cases, almost exclusively in rural areas of the developing world. It is generally believed to be underreported.[69] Similar to inhalational anthrax, gastrointestinal anthrax is more likely to be associated with bacteremia, sepsis, and seeding of other sites and, without antibiotics, is associated with a mortality rate of approximately 40%. Recognition and early treatment are crucial to survival, but because many victims are impoverished inhabitants in remote regions, antibiotics are often delayed until the disease has progressed to later stages.

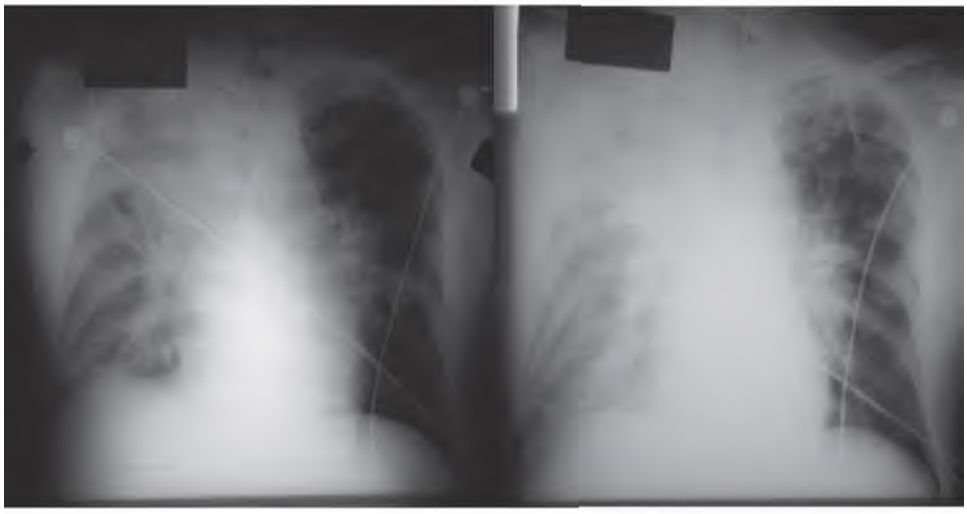
Most human cases are associated with the ingestion of undercooked meat (or uncooked dried meat) from an animal infected with anthrax, but a case in the United States was associated with a contaminated drum and probably demonstrates that spores deposited in the upper airways frequently are trapped in secretions and are eventually swallowed.[21] This is the only such case reported, and the patient had an associated *Enterobius vermicularis* infection of the small intestine and appendix. Large outbreaks in communities that have shared meat from dead animals have occurred, especially in Africa and Asia. In these settings, gastrointestinal anthrax cases may exceed the number of cutaneous cases.[69,70] Anthrax typically halts milk production in infected cows, but cow still producing have not been demonstrated to shed bacilli or spores in their milk. Furthermore, pasteurization kills vegetative *B. anthracis,* but not spores.[70] There have been no documented cases of natural infection from milk.

Typically symptoms develop 1 to 5 days after exposure for either oropharyngeal or intestinal disease, which may also be present concomitantly. In oropharyngeal anthrax, symptoms and signs of swelling, severe pharyngitis, dysphagia, and odynophagia at the site of inoculation in the mouth or pharynx; fever; and, in some cases, respiratory distress due to marked edema and lymphadenitis develop. An ulcer may be observed in the mouth or pharynx, and in one Turkish series it was localized to the tonsil in five of six patients and to the tongue in the sixth patient.[71] Pseudomembranes often form over the ulcer after the first week, bringing diphtheria into the differential diagnosis. Although significant neck swelling is seen in all oropharyngeal cases, massive facial and neck edema is occasionally seen. A peritonsillar abscess often is considered, but incision never yields purulent drainage. A swab of the base of oropharyngeal lesions typically reveals gram-positive bacilli and yields a positive culture.[72]

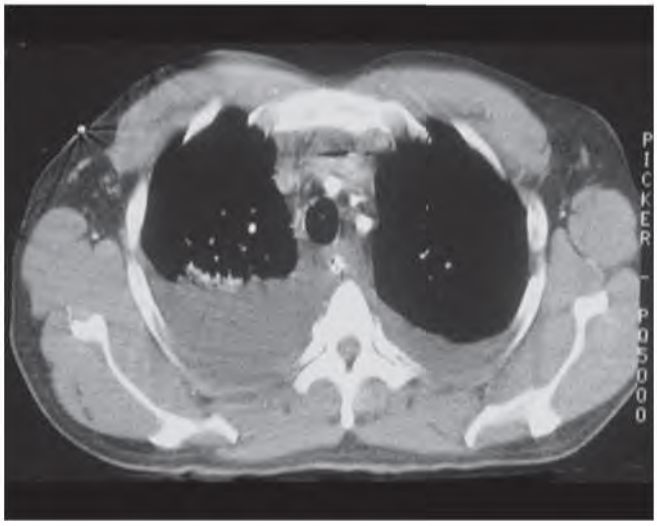
Intestinal disease occurs with infection of the stomach or bowel wall. The patient presents with nausea, vomiting, and fever, followed by severe abdominal pain that often manifests as a surgical abdomen.



10/21/01 0300 (intital ER visit) 10/22/01 0530 (hospital admit)



10/22/01 0900 10/22/01 1100 (shortly before death)



10/22/01 (shortly before death)

FIG. 207.7 Chest radiographs and computed tomography (CT) scan from a 47-year-old postal worker who had been ill for 5 days when he presented to the hospital with inhalational anthrax. Note progressive bilateral perihilar and infrahilar infiltrates, widened mediastinum, and rapid evolution. CT scan demonstrates mediastinitis and large right and smaller left pleural effusions. *ER,* Emergency room (department). *(Modified from Borio L, Frank D, Mani V, et al. Death due to bioterrorism-related inhalational anthrax.* JAMA. *2001;286:2554–2559.)*

Many cases are associated with hematemesis, massive ascites, and bloody diarrhea. Secondary meningitis is also common.

Table 207.1 outlines guidelines for diagnostic specimen preparation, handling, and testing. Blood, stool, and ascites samples all should be considered for culture and PCR testing.

Anthrax Meningitis

The frequency of anthrax meningitis is difficult to ascertain from published reports. Meningitis is an uncommon sequela of cutaneous anthrax but a frequent complication due to the bacteremia in inhalational or gastrointestinal disease, occurring in up to 50% of cases of the inhalational form. Although meningitis may rarely be the presenting symptom in some anthrax cases, it does not represent the initial site of infection (with the exception of a few case reports) and thus is not considered one of the primary forms of the disease.[73] In a review of reported anthrax cases from 1880–2013, 132 of 363 cases with systemic anthrax met criteria for anthrax meningitis. Severe headache, altered mental status, meningeal signs, and other neurologic signs at presentation independently predicted meningitis. Presence of even one of these factors had an 89% sensitivity for finding meningitis.[74]

As anthrax bacilli are released from macrophages into the bloodstream, the ensuing high-level bacteremia leads to seeding of other sites. The lethal and edema toxins have also been demonstrated to inhibit the innate immune responses of the blood-brain barrier in experimental infection, which may allow for easier access of the vegetative bacilli to the CNS.[75,76] Spread to the CNS may result from a focus of hemorrhagic necrosis that permits bacilli to pass to the meninges, cerebrospinal fluid (CSF), and brain parenchyma. The hallmark of anthrax meningitis is its hemorrhagic component associated with large gram-positive bacilli. CNS involvement may also include parenchymal brain hemorrhage and subarachnoid hemorrhage, possibly owing to a diffuse cerebral arteritis or necrotizing vasculitis. As might be expected from anthrax infections at other sites, cerebral edema may also be prominent.

Symptoms of meningitis usually occur in the presence of fulminant disease and are followed by death within 24 hours in 75% of cases. Initial symptoms include abrupt onset of severe headache, malaise, fever, chills, nausea, and vomiting. Meningeal signs such as nuchal rigidity may be absent early in the course but develop as the patient's condition deteriorates. Seizures, delirium, and coma usually follow within hours. Death was inevitable in the preantibiotic era but is currently estimated at approximately 95% of cases.[77] Early initiation of three or more antibiotics (focused on CNS penetration) has been associated with a greater potential for survival. Table 207.1 outlines guidelines for diagnostic specimen preparation, handling, and testing for anthrax meningitis. Blood and CSF should be obtained for stains, culture, and PCR testing.

THERAPY

Rapid initiation of appropriate antibiotic therapy is crucial in the treatment of anthrax, especially in the more severe noncutaneous cases. Table 207.2 outlines the oral treatment for cutaneous anthrax without evidence of systemic involvement. Table 207.3 outlines initial consideration for treatment of severe anthrax including anthrax meningitis.

Penicillin had been the drug of choice for all types of anthrax since the 1940s, but naturally occurring strains are increasingly reported to be resistant. However, *B. anthracis* is sensitive to a broad range of antibiotics including tetracyclines, macrolides, aminoglycosides, fluoroquinolones, carbapenems, linezolid, clindamycin, rifampin, quinupristin-dalfopristin, daptomycin, and first-generation (but not second- or third-generation) cephalosporins; chloramphenicol has demonstrated variable in vitro effectiveness in some studies.[78,79] In a study of 96 French anthrax isolates collected from the environment, animals, and one human case between 1994 and 2000, there was uniform resistance to third-generation cephalosporins, aztreonam, and trimethoprim-sulfamethoxazole. In 11.5% of the strains, penicillin and amoxicillin resistance and decreased sensitivity to second-generation cephalosporins were demonstrated, but there was nearly 100% sensitivity to the other 16 antibiotics tested.[80] Agents under study for their use in prophylaxis after spore exposure or treatment in clinical disease include oritavancin, cethromycin, daptomycin, and the novel inhibitor of the bacterial stringent response, Relacin, among others.[81–83] Several studies have demonstrated the combination of a bactericidal agent such as a penicillin, quinolones, or carbapenems with a protein synthesis inhibitor to be crucial in improving survival in animal models and the limited number of human cases reviewed.[84] An additional study also showed the combination of a bactericidal agent with a protein synthesis inhibitor was effective against a ciprofloxacin-resistant strain in an animal model.[85]

Most strains of naturally occurring *B. anthracis* have a chromosomally mediated, weak, inducible β-lactamase and cephalosporinase, and there have been rare reports of the development of resistance during therapy with penicillin, especially if subtherapeutic doses may have been administered. However, it is relatively easy to select for antibiotic-resistant strains of *B. anthracis* in the laboratory, and the resistance pattern of bioterrorist strains must be carefully assessed and therapy modified accordingly.[86,87] The guidelines for bioterrorism-associated anthrax recommend use of fluoroquinolones, carbapenems, and doxycycline until resistance testing is available, as β-lactam resistance in such strains is presumed to be likely.[88] Guidelines for treatment of severe anthrax, especially with meningitis, recommend use of three antibiotics—two bactericidal agents and a third protein synthesis inhibitor. All systemic anthrax infections should be treated with combination intravenous antibiotics for at least 2 weeks or until the patient is clinically stable, whichever is longer.[42,89]

TABLE 207.2 Oral Treatment for Cutaneous Anthrax Without Systemic Involvement

For all strains, regardless of penicillin susceptibility or if susceptibility is unknown
- **Ciprofloxacin, 500 mg every 12 hours**
  or
- **Doxycycline, 100 mg every 12 hours**
  or
- **Levofloxacin, 750 mg every 24 hours**
  or
- **Moxifloxacin, 400 mg every 24 hours**
  or
- Clindamycin, 600 mg every 8 hours[b]
  or

Alternatives for penicillin-susceptible strains
- Amoxicillin, 1 g every 8 hours
  or
- Penicillin VK, 500 mg every 6 hours

[a]Preferred drugs are indicated in **boldface**. Alternative drugs are listed in order of preference for treatment for patients who cannot take first-line treatment or if first-line treatment is unavailable. Duration of treatment is 60 days for bioterrorism-related cases and 7–10 days for naturally acquired cases.
[b]Based on in vitro susceptibility data, rather than studies of clinical efficacy.
From Hendricks KA, Wright ME, Shadomy SV, et al; Workgroup on Anthrax Clinical Guidelines. Centers for Disease Control and Prevention expert panel meetings on prevention and treatment of anthrax in adults. Emerg Infect Dis. *2014;20. doi:10.3201/eid2002.130687.*

Cutaneous Anthrax Without Systemic Manifestations

Naturally acquired (i.e., not occurring after an intentional release of spores) cutaneous anthrax with no evidence of systemic symptoms has been traditionally treated with oral penicillin for 7 to 10 days. As penicillin resistance appears in approximately 10% of naturally occurring strains of *B. anthracis* and older recommendations may lead to subtherapeutic dosing, the consensus of authorities convened by the CDC was that ciprofloxacin, doxycycline, levofloxacin, moxifloxacin, and clindamycin were the preferred first-line agents with amoxicillin or penicillin as alternatives once the sensitivity profile was determined (see Table 207.2). The duration of therapy for naturally acquired cases is 7 to 10 days. Unless there is clear evidence that an infection was naturally acquired, it should be considered to result from an intentional release with presumed concomitant inhalation of spores, and 60 days of treatment[42,90] with a fluoroquinolone or doxycycline is recommended as first-line therapy (ciprofloxacin, 500 mg orally twice daily, or levofloxacin, 750 mg daily, or doxycycline, 100 mg orally twice daily). If cultures were obtained from the lesions, modifications of the regimen may be made in response to the resistance profile seen. If systemic symptoms have developed,

TABLE 207.3 Intravenous Triple Therapy for Severe Anthrax With Possible or Confirmed Meningitis

1. A Bactericidal Agent (Fluoroquinolone)

Ciprofloxacin 400 mg q8h
or
Levofloxacin 750 mg q24h
or
Moxifloxacin 400 mg q24h
plus

2. A Bactericidal Agent (β-Lactam)
a. For All Strains, Regardless of Penicillin Susceptibility or if Susceptibility Is Unknown

Meropenem 2 g q8h
or
Imipenem[c] 1 g q6h
or
Doripenem 500 mg q8h
or

b. Alternatives for Penicillin-Susceptible Strains

Penicillin G 4 million units q4h
or
Ampicillin 3 g q6h
plus

3. A Protein Synthesis Inhibitor

Linezolid[d] 600 mg q12h
or
Clindamycin 900 mg q8h
or
Rifampin[e] 600 mg q12h
or
Chloramphenicol[f] 1 g q6–8h

Duration of Therapy

For 2–3 weeks or longer, until clinically stable. Will require prophylaxis to complete an antibiotic course of up to 60 days from onset of illness.

[a]Drug names in **boldface** are preferred agents. Alternative selections are listed in order of preference for therapy for patients who cannot tolerate first-line therapy or if first-line therapy is unavailable.
[b]Severe anthrax includes meningitis; inhalational, injectional, and gastrointestinal; and cutaneous with systemic involvement, extensive edema, or lesions of the head or neck.
[c]Increased risk for seizures associated with imipenem/cilastatin therapy.
[d]Linezolid may exacerbate thrombocytopenia; use for >14 days carries additional hematopoietic toxicity.
[e]Rifampin is not a protein synthesis inhibitor but may be used based on in vitro synergy.
[f]Should be used only if other options are not available, owing to toxicity concerns.
From Hendricks KA, Wright ME, Shadomy SV, et al; Workgroup on Anthrax Clinical Guidelines. Centers for Disease Control and Prevention expert panel meetings on prevention and treatment of anthrax in adults. Emerg Infect Dis. *2014;20. doi:10.3201/eid2002.130687.*

the patient should be treated with IV antibiotics as described for inhalational anthrax (see Table 207.3).

Cutaneous Anthrax (With Systemic Manifestations), Injectional Anthrax, Inhalational Anthrax, Gastrointestinal Anthrax, and Meningeal Anthrax

These frequently lethal forms of anthrax require aggressive management with multiple IV antibiotics, a vasopressor, a ventilator, and ICU support.[42] The use of antitoxin therapies such as AIG (Anthrasil; Cangene Corporation, Winnipeg, Canada) or one of the anti-PA MAbs raxibacumab (ABthrax; GlaxoSmithKline, Philadelphia, PA),[64] obiltoxaximab (Anthim; Elusys Therapeutics, Pine Brook, NJ), and Valortim (MDX-1303; PharmAthene/Medarex/Bristol-Myers Squibb, New York, NY) as well as investigational drugs should be considered in consultation with the CDC and other experts.[91,92] Patients frequently have an acute, rapid deterioration as anthrax infection progresses to the fulminant stages of bacteremia and, when possible, should be closely monitored in the ICU even if they initially appear clinically stable. Meningitis and brain parenchymal infection should be considered in all severe cases of anthrax because hemorrhagic seeding of the CNS occurs in approximately half of cases.[5,54]

Initial therapy should include two bactericidal agents and a protein synthesis inhibitor. If meningitis is suspected or confirmed, agents that have demonstrated adequate CNS penetration should be included in the regimen. CDC guidelines recommend an IV fluoroquinolone such as ciprofloxacin plus a carbapenem such as meropenem as the bactericidal agents. Protein synthesis inhibitors are added with the goal of diminishing bacterial toxin synthesis. Owing to its likely improved CNS penetration, the preferred protein synthesis inhibitor is linezolid, but clindamycin, rifampin, and chloramphenicol are alternative or additional recommended agents. Once antibiotic sensitivities of the *B. anthracis* isolate are known, penicillin may replace the carbapenem in the regimen. These guidelines with recommendations for all preferred and alternative agents are presented in Table 207.3.[42,88,89,90]

Owing to the critical need to use the most effective antimicrobial agents, the usual avoidance of quinolones and tetracyclines in children and pregnancy is superseded. As the resistance profile of the anthrax strain is determined and clinical improvement has been demonstrated, modifications of regimens can be made to diminish possible toxicity. Patients may also be transitioned to oral therapy as improvement occurs. Since the 2001 anthrax attacks, the recommendations for duration of therapy have been for a total of 60 days out of concern for delayed germination of inhaled spores. In most cases, a minimum of 10 to 14 days of IV therapy is required, followed by oral therapy. Data from studies in nonhuman primates with inhalational anthrax demonstrated that after a 10-day course of antibiotic therapy, begun after animals were bacteremic, the surviving animals developed an immune response and were protected against death from the delayed germination of retained spores even after discontinuance of antibiotic therapy.[63]

Management of Pleural Effusion and Ascites

Inhalational anthrax is associated with significant lymphatic obstruction leading to pulmonary edema and rapid accumulation of pleural fluid. Similarly, gastrointestinal anthrax may be associated with massive ascites. The 2001 anthrax cases demonstrated that the pleural fluid had the highest levels of anthrax bacilli as well as bacterial cell wall and capsular antigens.[67] PCR analysis for *B. anthracis* was most often positive from the pleural fluid.[50] After review of the cases of inhalational anthrax in 2001 and 2006 as well as the statistically significant decrease in mortality seen in the series of cases from 1990–2005, the consensus of experts is that early and aggressive management of pleural effusions with repeated thoracentesis or thoracostomy drainage is associated with increased survival.[19,54,67,68] In addition to the improved oxygenation afforded by minimizing loss of lung volume, study of toxin levels in the 2006 case demonstrated that pleural fluid has very high levels of LF.[93] This is essentially subjecting inhalational anthrax to the same standard of care as for an empyema or a complicated parapneumonic effusion. Ascites should also be continually monitored and drained because ascitic fluid can serve as a toxin reservoir, and significant fluid accumulations may further compromise pulmonary function.[94]

Role of Corticosteroids and Management of Severe Edema

The addition of corticosteroids has been advocated for treatment of cerebral edema and increased intracranial pressure in anthrax meningitis, based on its effect in improving outcomes in pneumococcal meningitis, although no controlled studies have been reported for anthrax. In addition, most recommendations for treatment of increased intracranial pressure include the use of hyperventilation and mannitol. Corticosteroid treatment has also been considered for the severe edema often associated with cutaneous, inhalational, and gastrointestinal cases resulting in life-threatening obstruction, massive pleural effusions, and ascites despite the lack of any controlled studies demonstrating efficacy.[95] Animal studies with corticosteroids and elucidation of the pathogenesis of fluid accumulation and meningitis are expected to give more objective evidence to support the use of corticosteroids in human anthrax.

Anthrax Antitoxin Therapies (Immunotherapeutics)

In the preantibiotic era, treatment of anthrax included incision, cautery, and application of acid. In the late 1890s French and Italian researchers developed animal-derived hyperimmune serum that eventually became the standard of care in North America by the 1920s and was used well into the 1950s. Antiserum was used to treat all forms of anthrax and was reported to have decreased the overall mortality rate of cutaneous cases from 24% to 6% in uncontrolled studies.[96] Serum preparations that were used and the quantity and routes of administration were almost arbitrarily determined, but case reports of survival of even bacteremic patients and lack of other effective treatments established antiserum as the treatment of choice. With the development of effective antibiotics, the use of antiserum gradually fell out of favor in most of the world, and it is no longer available in most countries. Anthrax antiserum is still available in some countries of the former Soviet Union and China, although it is seldom used.

Since the 2001 anthrax attacks, because of the high mortality associated with inhalational anthrax and the renewed appreciation of the role of the toxins in pathogenesis, there has been interest in the development of adjunctive therapies including antibodies. AIG is produced from plasmapheresis of individuals recently immunized with the licensed vaccine that consists mainly of PA. AIG was approved by the FDA in 2015 and is produced and marketed as Anthrasil under contract to the US government by Cangene Corporation. AIG was used in conjunction with antibiotics and ICU management for 19 patients: 3 with inhalational anthrax, 15 with injection anthrax, and 1 with gastrointestinal anthrax. All patients appeared to tolerate the antitoxin, and 2 of the patients with inhalational anthrax, 10 of the patients with injectional anthrax, and the 1 patient with gastrointestinal anthrax survived (Bower W, CDC, personal communication).[93,97]

Three human MAbs with high affinity for PA—raxibacumab,[64] obiltoxaximab, and Valortim (MDX-1303)—are currently approved by the FDA and are stockpiled by the US Department of Homeland Security. Other MAbs are also under development. One of the potential advantages of using MAbs, demonstrated in limited animal studies, is that their administration may not interfere with production of natural antibodies to anthrax.[98] Their half-life in humans after a single IV or intramuscular (IM) dose is approximately 4 weeks, with measurable antibodies for up to 2 months. The relative advantages of MAbs versus polyclonal antibodies remain to be determined in clinical studies, and polyclonal antibodies may be more effective for strains with PA resistant to a single MAb. Both are most effective in animal studies when given before or shortly after spore exposure but also have demonstrated efficacy in more advanced disease, especially where initiation of antibiotics may have been delayed.[98,99,100] In animal studies where antibiotics and antitoxin therapy were initiated at the onset of symptoms there was no advantage of combination therapy over antibiotics alone. However, when there was a delay in initiation of antibiotics after symptom onset, there was a survival advantage with combination therapy, but it did not reach statistical significance.[101]

The most experience with anthrax antitoxins in humans occurred with the recognition of injectional anthrax in people who used intravenous drugs in Scotland in 2009–10 where 15 recipients of AIG were compared retrospectively with 28 individuals who did not receive AIG. Death rates did not differ significantly between recipients of AIG and individuals who did not receive it, but the results were confounded due to the AIG recipients being sicker and at higher risk for death.[102]

In three human cases of inhalational anthrax treated with both antibiotics and antitoxin therapy, two patients survived and one died. Experts at the CDC who reviewed the three cases were unable to determine which of the intensive treatment measures was associated with survival. Antitoxin therapy blocks toxin translocation and does not affect toxin that has already entered cells. The patient who died developed respiratory symptoms earlier in the course of illness, and the CDC reviewers hypothesized that there may be a time point in disease progression after which intracellular damage is irreversible.[101] The CDC currently recommends use of anthrax antitoxin therapy in patients with all forms of anthrax who present with systemic symptoms. Although approved by the FDA, none of the antitoxin therapies is readily available without consultation with public health authorities at the CDC, Department of Defense, or state level. Experience in using the different antitoxin therapies is insufficient to recommend one as superior; expert consultation would help determine which product should be used.[42] The role of antitoxin therapy has not yet been defined in postexposure prophylaxis (PEP) but may be useful for strains of unknown antibiotic sensitivity in conjunction with antibiotics and immunization as soon after exposure as possible.[91,100,101,103]

PREVENTION

Vaccines against anthrax for both animals and humans have been used for more than 100 years, and effective use of live-attenuated veterinary vaccines has been associated with the decrease in animal and human cases in many regions of the world. Although there is some anecdotal evidence that immunity develops after infection based on the observation that human anthrax reinfections are rare and less severe, the best data are from studies in nonhuman primates demonstrating resistance to reinfection after recovery from inhalational anthrax.[104] Humoral immunity plays a critical role in the response to anthrax. Anti-PA is the most important antibody, and PA is the protective immunogen and the basis of the protection afforded by the human vaccines currently available. The role of the immune response to the other toxin components, EF and LF, in protection from anthrax infection is controversial, with conflicting studies demonstrating either additional protection or no added protection benefit when used to vaccinate animals despite adequate antibody responses.[105–107]

The currently approved US human vaccine, anthrax vaccine adsorbed (AVA) (BioThrax; Emergent BioSolutions Inc., Gaithersburg, MD), is a cell-free culture supernatant containing PA, derived from an unencapsulated, toxin-producing strain of *B. anthracis* that has been adsorbed to an aluminum hydroxide gel. The vaccine, developed by Wright and coworkers in the early 1950s, was licensed in 1970 and has been used for preexposure prophylaxis by veterinarians; laboratory, textile, and other workers who may be occupationally exposed; and the US military. PEP with AVA was approved by the FDA in 2015 and has been recommended by the US Advisory Committee on Immunization Practices to be used in conjunction with antibiotics for optimal management after exposure to aerosolized spores.[108]

In 2008 the FDA approved a modified five-dose regimen and administration route for preexposure AVA. The change from subcutaneous to IM administration decreases local side effects, and dropping the 2-week dose simplifies the regimen with no significant decrease in immunogenicity at 7 months, although antibody levels were significantly lower from 4 weeks to 6 months.[109] At 0 and 4 weeks, 0.5 mL of AVA is administered by IM injection, followed by subsequent injections at 6, 12, and 18 months as well as yearly boosters. For postexposure vaccination, a three-dose AVA regimen should be administered subcutaneously (in contrast to preexposure IM dosing) on a 0-, 2-, and 4-week schedule.

The vaccine approved in the United Kingdom, anthrax vaccine precipitated, is similar to AVA but is given as a four-dose series at 0, 3, and 6 weeks and 6 months, followed by annual boosters. Mild local side effects are as common with AVA as with other common adult vaccines, as are rare, idiosyncratic, serious side effects.[110] In a US Army study of 601 AVA recipients, 20% reported symptoms that they personally judged as mild enough to be ignored, 15% reported symptoms that affected their activity for a short time but did not limit their work duties, 8% reported short-term symptoms that were relieved with nonprescription medication, and only 2% reported symptoms unrelieved with medication and with short-term limitation of their work duties. Itching, subcutaneous nodules, and erythema were the most commonly reported symptoms, and reported symptoms were more common in women than in men.[111]

The safety of AVA has been the source of considerable controversy in relation to its mandatory use in the US military and objections to its side effects and purported long-term effects. However, the safety and efficacy of AVA were confirmed in an extensive review by the Institute of Medicine of the National Academy of Sciences in 2002[112] as well as additional safety studies since then.[113] No serious adverse events were noted in a review by the CDC of individuals given the vaccine as PEP

after the 2001 anthrax events.[114] As a cell-free filtrate, AVA cannot cause anthrax. Its use has not been associated with birth defects, and a possible association with optic neuritis and multiple sclerosis was shown not to be statistically significant.[112,115]

A live-attenuated vaccine consisting of spores from an unencapsulated toxigenic strain of *B. anthracis* has been used in the former Soviet Union since 1953. The vaccine, given via scarification or subcutaneously, is said to be reasonably well tolerated and is reported to afford some degree of protection against cutaneous anthrax.[111] The Chinese developed a similar live spore vaccine in the 1960s.

Since the 2001 anthrax attacks there has been an intense effort to produce newer anthrax vaccines with a less cumbersome dosing schedule and less local reactogenicity. Several highly purified, recombinant PA-based vaccines adjuvanted with aluminum hydroxide (and other novel adjuvants) have been shown to protect nonhuman primates against inhalational anthrax with only one or two doses and are undergoing clinical trials.[116–118] Additional efforts to improve PA-based vaccines include using PA with other adjuvants and delivery systems including DNA vaccines and given by different routes. Research has also identified additional antigens as potential future vaccine candidates such as the anthrax capsule,[119,120] spore proteins,[121] and others that have been shown to be protective in experimental animals.[111,122]

ANTHRAX AS AN AGENT OF BIOTERRORISM

When bioterrorism became a reality in the autumn of 2001, worldwide interest became focused on *B. anthracis,* a bacterium associated with what had become a relatively obscure disease of the developing world. With a few grams of anthrax spores dispersed in letters, the recognition of the threat of bioterrorism prompted a dramatic increase in research, training, public health preparedness, countermeasures, and response infrastructure. The general level of understanding of all the agents of bioterrorism was raised not only in the medical community but also among first responders, legislators, and the general public.

Anthrax remains the agent of greatest concern for future use as a bioterrorist's weapon. With naturally occurring cases not uncommon in much of the world, *B. anthracis* is readily accessible to terrorists; easy to grow in even a rudimentary laboratory; and, in the spore form, stable, easily stored, and portable in small quantities that can wreak havoc when dispersed.

History of *Bacillus anthracis* as a Bioterrorist Agent

The history of anthrax being spread intentionally to infect others is relatively recent (compared with plague and smallpox), extending only as far back as World War I, when Germans were reported to have shipped infected horses and cattle with *B. anthracis* to be used by the Allies.[123] A more bizarre story is that of Finnish independence activist Baron Otto Karl von Rosen, who was apprehended by Norwegian police in 1917 with 19 sugar cubes, each with an embedded glass capillary tube supposedly filled with anthrax. Apparently, the plan was to infect reindeer and horses used to haul British arms through Norway. The sugar cubes would be fed to the animals, whose teeth would break the glass tubes inside, thereby lacerating their gums and allowing oropharyngeal or gastrointestinal anthrax to ensue.

During World War II, both the Axis and the Allies had biological warfare programs that involved anthrax, including the British, whose spore bomb experiments on the Scottish Gruinard Island rendered areas of the island heavily contaminated for decades.[124] The Japanese carried out extensive research on biological weapons. They used anthrax-infected animals to spread the disease in Russia and intentionally infected Chinese residents of Manchuria and China by various means including an unsuccessful attempt to infect children with anthrax-laden chocolate.[125]

Beginning in the 1940s the United States maintained an offensive biological warfare program that performed numerous studies on anthrax weaponization and defense that remain the basis of much of our understanding of bioterrorism today. The offensive program at Fort Detrick, Maryland, was disestablished by President Nixon in 1969 and replaced by the US Army Medical Research Institute of Infectious Diseases, which has been on the forefront of biodefense for more than 40 years.

The Soviet Union maintained an active anthrax program well into the 1990s. The widely studied accidental release of anthrax spores in 1979 from a Soviet military microbiology facility in Sverdlovsk, Russia, was responsible for approximately 70 human cases of inhalational anthrax.[5,126] It remains the largest outbreak of inhalational anthrax known.

During the reign of Saddam Hussein, Iraq was known to have an active biological warfare program, and 16 other nations were suspected of having biological warfare programs, but it is unknown which may have been working with anthrax.[127] At the close of the first Gulf War, Iraqi authorities admitted to having produced 8500 L of anthrax and placed 6500 L into munitions but denied ever having used them.[6,128]

Far more widely known is the use of the US Postal Service in 2001 to mail anthrax spore–laden letters, after which 22 people developed anthrax, 11 with inhalational infections and 11 with cutaneous infections. There were five deaths among the inhalational cases. An astute infectious diseases physician first considered the diagnosis when a Florida man was noted to have gram-positive rods in his CSF. Subsequent letters were sent to recipients in New York City and Washington, DC, including media outlets and offices of Senators Thomas Daschle and Patrick Leahy in the US Capitol.

The epidemiology of the 2001 anthrax cases completely changed understanding of the dispersal of anthrax spores. The ease with which spores were released from sealed envelopes during mail handling was a startling development that was not recognized until hospitalization of postal employees with inhalational anthrax occurred in individuals never suspected to have been exposed. Approximately 10,000 Americans subsequently received anthrax PEP with antibiotics because of possible exposure. The extensive cross-contamination of mail that led to additional cases geographically distant from the source was a completely unpredicted finding.[129,130] Finally, the substantial reaerosolization of spores from surfaces was not anticipated from studies performed decades previously and contributed to the $100 million spent on anthrax remediation (decontamination) in the United States, with $23 million spent at the US Capitol complex alone.[125–131]

The anthrax events of 2001 fueled a massive investment by the US government in broad areas of research on the agents of bioterrorism.[132] Since these events, new rapid, sensitive, and specific diagnostic tests have become available and are dispersed regionally as part of the Laboratory Response Network system of laboratories with varying biosafety levels (BSLs): sentinel (BSL-2), reference (BSL-3), and national (BSL-4) laboratories. Extensive bioterrorism educational efforts were made with physicians, nurses, infection control practitioners, and first responders. The intensive search for the source of the anthrax spores drove rapid improvements in microbial forensics, and the decontamination efforts in postal facilities, the US Capitol, and multiple media outlets advanced the science behind environmental remediation.[133–135] The anthrax events, occurring soon after the terrorist attacks of September 11, 2001, provoked a sense of urgency in both legislators and the scientific and medical communities that has led to considerable improvements in countermeasures such as antibiotics, therapeutic antitoxin antibodies, and vaccines. Project BioShield was created in 2004 in the United States to provide the Strategic National Stockpile funds to maintain a supply of antibiotics and other therapeutics, vaccines, and diagnostics in the event of a national emergency. Furthermore, BioShield not only provides funding for phase III clinical trials to obtain approval by the FDA but also gives the option of using supplies that may not have been fully approved by the FDA if the CDC declares an emergency.[136] The Biomedical Advanced Research and Development Authority was subsequently created by the US Congress to fund advanced development of countermeasures and oversee BioShield contracts.

Dissemination of Anthrax as a Bioterrorist Agent

History has already presented a number of methods in which anthrax can be weaponized. Anthrax has proven itself to be a versatile agent for a terrorist to use. Spores can be dispersed or sprayed as a powder or liquid, or animals can be infected and released with the intent to spread infection among others. Anthrax can be delivered by an aerosol in bombs, sprayed from a plane or backpack sprayer, or sent in the mail. It is generally believed that an intentional release of anthrax would

most likely be associated with aerosols and subsequent inhalational infections. Although contamination of human food or water could cause gastrointestinal anthrax, evidence from experimental animals suggests that it is not an efficient route and requires much higher doses of spores to lead to infection.[137] Anthrax is also an efficient agent; the 2 g of spores that were in the letter sent to Senator Thomas Daschle in 2001 were estimated to contain more than 10 million human LD_{50} (lethal dose for 50% of individuals).[138]

In a report to the US Congress, it was estimated that 100 kg of anthrax spores released from a plane over Washington, DC, would kill 1 to 3 million people under ideal meteorological conditions.[137] Other studies indicated there could be 50% fatalities as far as 160 km downwind from an aerosol spore release.[139]

A 1999 Canadian study that investigated the effect of aerial spraying of *Bacillus thuringiensis* for control of gypsy moths in British Columbia was indicative of why anthrax is considered such a high-threat agent. *B. thuringiensis* is a spore-forming species closely related to *B. anthracis* but a nonpathogen in animals. A plane sprayed a slurry of spores at a droplet size of 110 to 130 µm, and samples were taken from the environment, inside homes, and from nasal swabs of asthmatic children. Thirty minutes after release, spore concentrations were highest in the outdoor environment (mean, 739 spores/m^3 of air), but 5 or 6 hours later spore concentration inside homes exceeded outdoor concentrations (mean, 245 spores/m^3). A significant amount of small droplet aerosolization occurred with droplets of 2 to 7 µm formed in sufficient quantities to penetrate houses, yielding positive nasal swabs in 76%. (Droplets <5 µm can reach the alveoli.) If this had been *B. anthracis,* some estimates are that potentially 15% of the population sprayed may have been exposed to a lethal dose of anthrax.[140] Furthermore, the spray formulations and equipment used are readily available to the public through agricultural supply stores, and these formulations do not clog spray nozzles.[141]

Determinations of the number of inhaled spores per hour raise questions about the infective dose (ID) and lethal dose (LD) of anthrax spores. Obviously, controlled studies cannot be performed on humans; therefore we must rely on extrapolation of data from estimates of known inhalational cases and from studies in nonhuman primates. Even this is difficult because different animals have markedly different sensitivities to anthrax spores: some are very sensitive and others are quite resistant.[59,142] Despite the uncertainty inherent in extrapolation, the LD_{50} for humans is generally considered to range from approximately 4000 to 55,000 spores based on studies in nonhuman primates.[31,59,104,143] Data from nonhuman primates suggest that inhaled doses of 1000 to 5500 spores results in mortalities from 10% to 25%.[144] The determination of the LD_1 (or ID_1) is even more difficult to ascertain, and it may be significantly lower, but there are no data on this point. From a public health perspective, even an LD_1 or LD_{10} release may be significant because a city of 1 million might experience tens of thousands of cases of anthrax.[140]

Studies from mill workers in the 1950s and 1960s revealed that many employees were inhaling hundreds of anthrax spores less than 5 µm on a daily basis, and yet inhalational anthrax was extraordinarily rare.[29,145,146] Furthermore, 15% of workers developed measurable anti-anthrax antibodies, demonstrating some degree of exposure by an unknown route, suggesting some degree of innate immunity in avoiding clinically evident infections.[57] Even in the absence of measurable antibodies, exposures in the US Capitol appear to have been associated with evidence of a cellular immune response in a few cases.[147] Historically, some individuals with inhalational anthrax had evidence of preexisting pulmonary disease, which may have made them more susceptible.[144,148] What may be a "safe" number of spores for a healthy 20-year-old may be a lethal dose for an immunocompromised individual.

Outbreak Characteristics After Use of Anthrax as a Bioterrorist Agent

A terrorist considering using anthrax may contemplate which route to deliver spores to reach his or her objective. Although spores could be introduced into food supplies or water, the cutaneous, oropharyngeal, and gastrointestinal disease that would result is far less dramatic and far less fatal than inhalational anthrax.[138] Knowledge of the number of spores needed to infect humans via ingestion is not available. It is generally understood that significantly more are needed than via inhalation, and experiments in nonhuman primates suggest it is very difficult to deliberately cause infection by the oral route. In addition, the majority of ingestions are not associated with full-blown gastrointestinal anthrax, which is frequently fatal, but rather with gastroenteritis, a far less dramatic presentation than inhalational anthrax.[69]

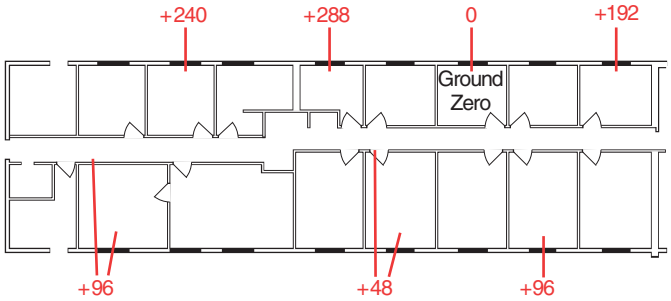


FIG. 207.8 Spores released in an office spread very rapidly through doors and in hallways. Time in seconds for peak spore deposition at a site after release of spores from an envelope at "ground zero." *(From Kournikakis B, Ho J. Objective assessment of the hazard from anthrax terrorist attacks in an office environment. Presented at the Anthrax Incident Management Workshop, Medicine Hat, Alberta, Canada. April 30, 2002.)*

An aerosol release, whether from an envelope, a sprayer on the ground, or on a larger scale from a plane, is considered the most likely terrorist scenario. Studies done in Canada with envelopes containing *Bacillus globigii (Bacillus atrophaeus)* spores demonstrated how spores spread like a gas in an office after opening an envelope containing 1 g of spores (Fig. 207.8). The estimates were that with 10 minutes of exposure, hundreds to thousands of times a human LD_{50} would be inhaled by people in the room.[149,150]

Individual *B. anthracis* spores are 1.5 to 3 µm; in nature they are most likely clustered together to form aggregates that are 10 to 100 µm in diameter or greater. As Fig. 207.9 demonstrates, particles larger than 5 µm typically cannot reach the terminal bronchioles and alveoli; they are captured in the respiratory tract mucus, removed by the mucociliary elevator to the mouth, and swallowed.[151] This is likely why inhalational anthrax has been uncommon in natural settings but was more common in factories where wool, hair, or hides were dried and manipulated by machinery, resulting in particle sizes of 1 to 5 µm. When spores are grown and engineered in a laboratory with the intent of preventing clumping by coating with silica or other substances, they may not cluster at all or may form small spore aggregates that may be efficiently delivered to the alveoli. It can be assumed that a sophisticated terrorist using anthrax spores would consider the following:

1. Engineering of spores
   a Enhancement of stability and infectivity
   b Neutralization of electrostatic charges, thereby reducing clusters and maintaining spores in small respirable aggregates less than 5 µm
2. Use of high concentrations of spores to overcome any degree of innate immunity
3. Selection of a strain demonstrating antimicrobial resistance
4. Genetic modifications to decrease protection from vaccination or increase toxin production

When anthrax spores pass the terminal bronchioles into the alveoli, they are rapidly scavenged by alveolar phagocytic cells or are translocated across alveolar epithelial cells and move through the lymphatics to the tracheobronchial lymph nodes and then mediastinal lymph nodes. Some spores rapidly transform to the vegetative state within the macrophages, whereas others are thought to remain quiescent potentially for months.

The incubation period in natural inhalational anthrax is generally considered to be 2 to 10 days, but with large inocula it may be as short as 1 day. In the 2001 cases for which the date of exposure was known, the incubation period was 4 to 6 days.[68] There is some controversy regarding how long the incubation can potentially be and whether small spore inocula may be associated with extended incubation periods.[29,129,144] The often-cited last case of anthrax in Sverdlovsk occurred 43 days after

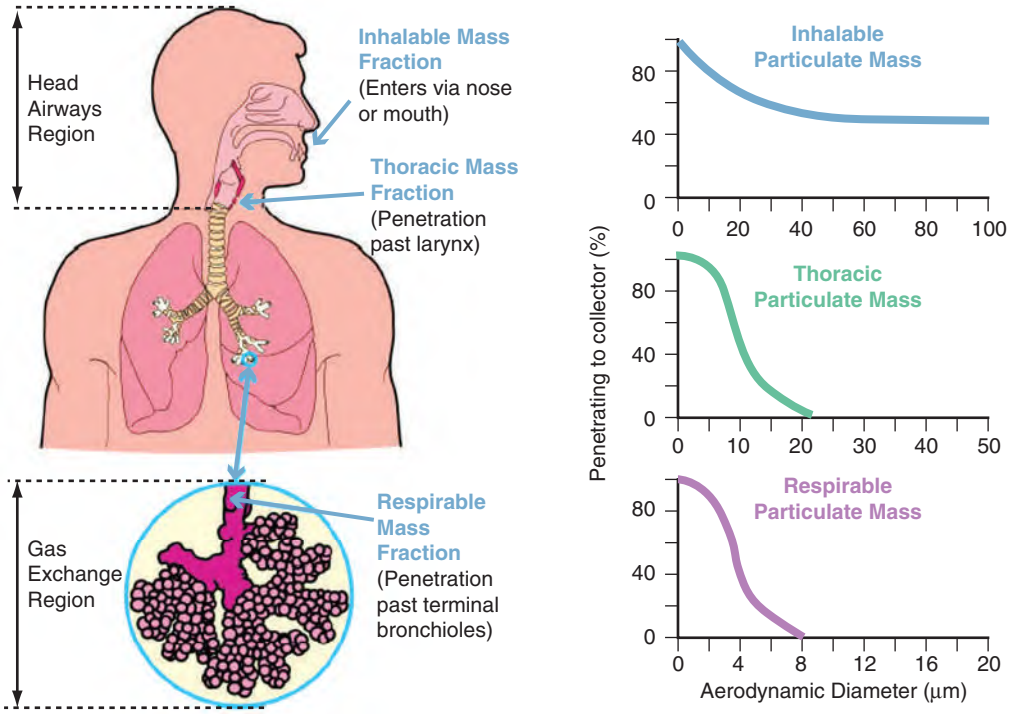


FIG. 207.9 Deposition of anthrax spores in the human respiratory tract depends on the size of spore aggregates. Individual spores are 1.0 to 1.5 μm but form aggregates 10 to 100 μm or larger that are deposited in the upper airways and cleared; weaponized spores may not cluster and are efficiently delivered to the alveoli, where they may lead to inhalational anthrax. *(Modified from Hoover MD. Uncertainty and probability distribution analyses for anthrax dispersion and human exposure. Presented at the Anthrax Incident Management Workshop, Medicine Hat, Alberta, Canada. April 30, 2002.)*

the release of spores, but median incubation for those closest to the release was 10 days and for those farthest away was 21 days.[126] However, these data are of dubious value because crucial details are not available, and patients may have received antibiotics, which are known to extend the incubation period. The case of the 94-year-old woman in Connecticut who developed anthrax in 2001, presumably related to the terrorism cases that year, occurred 35 days after the last letter was mailed and 56 days after the first, suggesting she may have inhaled a small dose. However, the possibility of exposure resulting from a different unrecognized intentional release of spores cannot be excluded.[129] In a series of 58 nonhuman primates, one of us (A.M.F.) noticed the time to death after exposure to spores varied from 2 to 9 days. However, there are reports of three infected and untreated animals with possible times to death of 20, 28, and 98 days after exposure.[29,31] Animals sacrificed 42 days after inhalation had a large number of spores in the lungs; after 100 days, small numbers of viable anthrax spores remained.[31]

The ability to detect anthrax spores soon after an aerosol release or to diagnose the first case of anthrax has improved significantly in the past decade. In a large spore release over a city, every day earlier that the exposure is recognized will result in earlier initiation of PEP and thousands fewer cases and millions of dollars in saved resources.[152,153] Even with an extensive system of detection in place, it is likely that the first evidence of anthrax bioterrorism, as in 2001, will be a critically ill patient discovered to have *B. anthracis* in a blood culture. Again, as in 2001, it can be expected that some patients will present with cutaneous disease (because aerosolized spores deposited on the body can be introduced into the skin) or gastrointestinal disease (as demonstrated in the American woman at an animal hide drumming event),[21] and others will present with inhalational disease or meningitis. If an extensive outbreak is recognized, local medical resources will be severely taxed attempting to initiate PEP, evaluating individuals potentially developing symptoms, and caring for individuals already confirmed infected and potentially critically ill. The past decade has been marked by numerous studies modeling the effects of an anthrax spore release and the subsequent response by government, health care institutions and facilities, and private individuals.[154,155]

Although there are numerous rapid assays in development, there is currently no rapid method approved to diagnose anthrax early in disease. The early symptoms of inhalational anthrax are nonspecific and similar to those of influenza. It is important to rapidly determine who has inhalational anthrax, influenza or influenza-like illness, or community-acquired pneumonia so that appropriate therapy can be initiated.[65,156,157] Kuehnert and associates[157] combined data from patients presenting with each of these diagnoses and developed a scoring system. They found that compared with patients who had influenza-like illness, patients who had inhalational anthrax were more likely to have tachycardia, high hematocrit, low albumin, and low sodium levels and were less likely to have myalgias, headache, sore throat, and nasal symptoms. Compared with patients who had community-acquired pneumonia, patients with inhalational anthrax were more likely to have nausea or vomiting, tachycardia, high aminotransferase levels, low sodium levels, and normal white blood cell counts (Tables 207.4 and 207.5).[157] The use of rapid influenza and respiratory syncytial virus diagnostics in the clinic or emergency department is also indicated. Patients with fever and spore exposure should also have blood cultures obtained; all seven patients with inhalational anthrax in 2001 who were not taking antibiotics had positive blood cultures. Kyriacou and coworkers[158] compared 47 inhalational anthrax cases with 376 community-acquired pneumonia or influenza-like illness cases and found the most accurate predictor of anthrax was a chest radiograph demonstrating mediastinal widening or pleural effusion. The epidemiologic as well as diagnostic significance of mediastinal widening needs to be emphasized in recognizing aerosol route of exposure to anthrax. It has been erroneously stated[159] and repeated in the literature[70] that mediastinal widening was reported to occur in a case of gastrointestinal anthrax. However, a careful reading of the original article reveals no mention of mediastinal widening, but rather that pneumonia was present on chest radiography, which the authors believed to be secondary to bacteremia. This is consistent with our ideas of pathogenesis in that the lymph nodes draining the site where the spores are introduced are those anticipated to become infected and enlarged. Thus mediastinal widening on a radiograph should alert the physician to suspect inhalational anthrax from an aerosol exposure

TABLE 207.4 Signs and Symptoms of Patients Presenting With Inhalational Anthrax, Influenza or Influenza-Like Illness, and Community-Acquired Pneumonia

SIGN OR SYMPTOM	INHALATIONAL ANTHRAX (*N* = 11), %	INFLUENZA OR INFLUENZA-LIKE ILLNESS (*N* = 684), %	COMMUNITY-ACQUIRED PNEUMONIA (*N* = 650), %
Tachycardia	82	14 (*P* = .0001)	49 (*P* = .04)
Sore throat	18	76 (*P* = .0001)	25 (*P* = 1.0)
Nasal symptoms	27	81 (*P* = .0002)	34 (*P* = .76)
Headache	45	86 (*P* = .002)	38 (*P* = .76)
Myalgias	64	91 (*P* = .01)	41 (*P* = .21)
Fever >37.8°C (100°F)	73	57 (*P* = .37; with flu *P* < .05)	53 (*P* = .23)
Cough	91	89 (*P* = 1.0)	79 (*P* = .47)
Fatigue	100	98 (*P* = 1.0)	NA
Abdominal pain	27	NA	21 (*P* = .71)
Diarrhea	9	NA	20 (*P* = .70)
Nausea or vomiting	82	NA	35 (*P* = .002)
Chest pain	64	NA	31 (*P* = .04; if pneumococcal bacteremia *P* > .05)
Dyspnea	82	NA	80 (*P* = 1.0)
Chills	82	NA	59 (*P* = .21)

NA, Not applicable.
Modified from Kuehnert M, Doyle T, Hill H, et al. Clinical features that discriminate inhalational anthrax from other acute respiratory illnesses. Clin Infect Dis. *2003;36:328–336.*

TABLE 207.5 Laboratory Findings in Patients Presenting With Inhalational Anthrax, Influenza or Influenza-Like Illness, and Community-Acquired Pneumonia

LABORATORY PARAMETER	INHALATIONAL ANTHRAX (*N* = 8–11), %	INFLUENZA OR INFLUENZA-LIKE ILLNESS (*N* = 630–687), %	COMMUNITY-ACQUIRED PNEUMONIA (*N* = 185–645)
Leukocytosis	27	7 (*P* = .04; without confirmed flu, *P* > .05)	61 (*P* = .03)
Neutrophilia	72	43 (*P* = .06; without confirmed flu, *P* < .05)	78 (*P* = .71)
High hematocrit	36	6 (*P* = .004)	NA
Low platelets	22	4 (*P* = .05)	NA
High bilirubin	38	6 (*P* = .009)	NA
High AST	89	18 (*P* < .0001)	29 (*P* = .0004)
High ALT	89	32 (*P* = .0008)	29 (*P* = .0005)
Low albumin level	67	2 (*P* < .0001)	NA
Low sodium	80	9 (*P* < .0001)	35 (*P* = .0005)
High BUN	50	4 (*P* < .0002)	23 (*P* = .10)
High creatinine	0	1 (*P* = 1.0)	NA
Low potassium	10	2 (*P* = .21)	NA
Low calcium	100	44 (*P* = .002)	74 (*P* = .21)

ALT, Alanine aminotransferase; *AST,* aspartate aminotransferase; *BUN,* blood urea nitrogen; *NA,* not applicable.
Modified from Kuehnert M, Doyle T, Hill H, et al. Clinical features that discriminate inhalational anthrax from other acute respiratory illnesses. Clin Infect Dis. *2003;36:328–336.*

and a bioterrorist event until proved otherwise. The evolution of chest radiographs and CT in inhalational anthrax is demonstrated in US cases from 2001 as shown in Fig. 207.7.[160] The Center for Infectious Disease Research and Policy has developed a helpful clinical pathway to guide clinicians assessing the probability of anthrax exposure and evaluating patients with possible inhalational anthrax (Fig. 207.10 and Table 207.6).[27]

ANTHRAX COUNTERMEASURES

Diagnostics

It is important to notify the laboratory that anthrax is in the differential diagnosis because the typical clinical laboratory may discard gram-positive rods as probable contaminants or not work them up beyond *Bacillus* spp. Furthermore, if *B. anthracis* is isolated, it should be handled in at least a BSL-2 laboratory (i.e., under a hood) because secondary cases have occurred among laboratory workers.

The frequency of encountering other *Bacillus* spp. has been part of the problem in developing sensitive and specific rapid tests for anthrax from both environmental and clinical sources. When Dahlgren and coworkers[146] reported on *B. anthracis* aerosols in goat hair mills, they noted the difficulty in finding the organism because it was obscured by other spore-forming bacteria in a ratio of 115:1 to 700:1. Recently developed, more specific PCR assays have become available that should aid in rapid diagnosis and minimize the number of false-positive samples.[49]

The role of nasal swabs in the "diagnosis" of anthrax must also be clarified. In the 2001 outbreak, patients considered nasal swabs as a determination of whether they had been exposed or not. The reality is that although a positive nasal culture for anthrax clearly indicates an exposure, a negative culture does not rule out an exposure. In Senator Thomas Daschle's suite in the Hart Building of the US Capitol, all 13 staff members in the room where the spore-laden letter was opened had heavy growth on blood agar plates from their nasal swabs. Many of these individuals had a separate swab from each nostril, and in at least two cases, one of us (G.J.M.) noted that one nostril of the individual yielded heavy growth and the other nostril yielded no *B. anthracis* growth. Nasal swabs essentially use the nose to sample whether the individual has filtered anthrax spores in the recently (nasally) inhaled air. They are therefore helpful as a public health tool in determining the zone of exposure. Demonstrating that one individual in a space has a positive nasal swab requires that everyone in that space receive PEP regardless of negative nasal swabs for the others. The optimal timing of obtaining nasal swabs after exposure has not been determined, but clearly the sooner the better, and it is likely that the yield 24 hours later (e.g., after showering) is much lower. Thus obtaining nasal swabs more than 24 hours after exposure should be discouraged. All 28 positive cultures in the US Capitol were obtained within hours of exposure; the remaining 6000 cultures done during the subsequent days were negative despite environmental samples demonstrating varying levels of contamination from multiple other US Capitol sites. One of us (G.J.M.) observed that repeat nasal swabs of all the culture-positive individuals in the US Capitol 1 week after exposure were negative. In evaluation of future exposures, efforts to determine if an individual was exposed might also include culturing pharyngeal washings because a study of wool mill workers revealed that addition of such cultures doubled the number of individuals with positive cultures compared with culturing only nasal swabs.[145] Gram stain of cutaneous or oral lesions, pleural fluid, CSF, or even buffy coats of blood may be positive for gram-positive rods indicative (in the right setting) of anthrax, and culture will confirm the diagnosis. Nasal swab, pharyngeal washes, and stool samples should be cultured for anthrax, but Gram staining of these samples is not helpful.

In the 2001 outbreak, there were no serologic tests readily available for anthrax. In the aftermath, numerous serologic assays have been in development and a number of rapid ELISAs that measure total antibody