functional TLR4 response are highly susceptible to *Salmonella* infection, confirming the importance of this initial response to infection.[130] Other studies indicate that caspase-1 and caspase-11, proteases important for secretion of IL-1 and IL-18, as well as inflammatory cell death in host cells, are important for infection in mice by the oral and systemic route, indicating that recognition and activation of the cytoplasmic sensing components by NOD family members are important for control of infection in the intestinal mucosa.[131] Recent evidence indicates this cell death and secretion of cytokines is mediated by inflammatory caspases cleaving proteins called gastrodermins, which leads to specific membrane pore formation through which cytokine release and cell death occurs.

Studies in mice further demonstrate that the initial control of *S.* Typhimurium replication in host tissue requires recruitment and activation of macrophages. In both mice and humans, macrophage activation and efficient killing of *Salmonella* are associated with production of interferon-γ (IFN-γ), IL-12, and tumor necrosis factor (TNF)-α.[132–134] Mice with targeted disruptions in the genes for these molecules are highly susceptible to infection. Rheumatoid arthritis patients treated with TNF antagonists have developed severe and fatal septicemias.[135] In addition, humans with mutations in the IFN-γ and IL-12 receptor genes develop severe infections with NTS serotypes.[136]

Although the innate immune system is able to suppress initial *Salmonella* replication, final clearance of infection and immunity to rechallenge requires a Th1-type CD4 T-cell response and production of specific antibodies by B cells.[134] *Salmonella*-specific antibodies are needed to control extracellular replication of *Salmonella* in the blood and are absent in most healthy Malawian children younger than 16 months, potentially contributing to the observed high rates of NTS bacteremia in African children younger than 2 years.[137] Both antibody and complement are required for induction of oxidative burst killing and phagocytosis of some African strains of NTS by neutrophils and monocytes.[138] Mice lacking mature CD4 cells (H2I; AB^- mice) or B cells ($Igh\text{-}6^-$ mice) are unable to control *Salmonella* infection.[134,139,140] The importance of cellular immunity in controlling *Salmonella* infection in humans is made apparent by the extreme susceptibility of individuals with HIV infection, lymphoproliferative diseases, or immune suppression after transplant.[141,142] A variety of other immunodeficiencies have been associated with *Salmonella* infection, including common variable immunodeficiency.[143] Furthermore, vaccine studies in humans demonstrate that protection against *S.* Typhi infection correlates with development of cell-mediated immunity.[144] In accord with the importance of CD4 T-cell–mediated immunity, population-based studies in humans have found an association between specific class II major histocompatibility complex alleles and susceptibility to typhoid fever.[145] CD8 T cells with cytolytic activity against *Salmonella*-infected host cells are also present in infected mice, but the importance of this activity in immunity remains unclear. Little is known about the antigen specificity of protective immune responses to *Salmonella* infection in humans, although antibodies against the Vi polysaccharide, LPS O antigen, and flagella are present in previously vaccinated or infected individuals.

CLINICAL MANIFESTATIONS

Specific *Salmonella* serotypes most often produce characteristic clinical syndromes, including gastroenteritis, enteric fever, bacteremia and vascular infection, localized infections, and the chronic carrier state, and outcomes, including case-fatality rates and hospitalization rates, differ substantially by serotype.[146]

Gastroenteritis

Infection with NTS most often results in self-limited acute gastroenteritis that is indistinguishable from that caused by many other enteric bacterial pathogens. Typically, within 6 to 48 hours after ingestion of contaminated food or water, nausea, vomiting, and diarrhea develop, but median incubation periods of up to 7 days have been reported from foodborne outbreaks of *S.* Enteritidis, likely correlating with low ingested dose.[147,148] In most cases, stools are loose, of moderate volume, and without blood. In rare cases, stool may be watery and of large volume ("cholera-like") or of small volume associated with tenesmus ("dysentery-like"). Fever (38°–39°C), abdominal cramping, nausea, vomiting, and chills frequently are reported. Headache, myalgias, and other systemic symptoms also may occur. Microscopic examination of stools shows neutrophils and, less frequently, red blood cells. NTS can cause a syndrome of pseudoappendicitis, can mimic the intestinal changes of inflammatory bowel disease, or rarely causes toxic megacolon.[147,149]

Gastroenteritis caused by NTS is usually self-limited. Diarrhea resolves within 3 to 7 days and fever within 72 hours.[147] Persistent NTS infection with relapsing diarrhea lasting up to 8 years has been described in a small fraction of patients and was associated with in-host single nucleotide mutations in key virulence regulators.[150] On occasion, patients require hospitalization because of dehydration, and death occurs infrequently. In the United States, NTS infections result in an estimated 19,336 hospitalizations and 378 deaths per year.[16] A disproportionate number of these deaths occur among the elderly, especially those residing in long-term care facilities, and among immunocompromised patients, including persons with HIV/acquired immunodeficiency syndrome (AIDS), stem cell transplantation, or those who are receiving biologic therapies.[151,152]

After resolution of gastroenteritis, the mean duration of carriage of NTS in the stool is 4 to 5 weeks in adults and 7 weeks in children.[153] Longer duration of stool carriage has been associated with random single nucleotide mutations in *S.* Typhimurium strains.[154] Antimicrobial therapy may increase the duration of carriage.[155] Acute NTS gastroenteritis has been associated with an increased risk of developing irritable bowel syndrome in several surveillance and outbreak studies with methodologic limitations.[156]

Enteric Fever

Enteric fever is a severe systemic illness characterized by fever and abdominal pain that is caused by disseminated infection with *S.* Typhi and *S.* Paratyphi A, B, and C. The definitive diagnosis of enteric fever requires the isolation of *S.* Typhi or *S.* Paratyphi from blood, bone marrow, another sterile site, intestinal secretions, or from punch biopsy of rose spots—the faint salmon-colored maculopapular skin lesions on the trunk stool that may appear at the end of the first week of illness (see Chapter 100).

Bacteremia and Vascular Infection

Up to 8% of patients with NTS gastroenteritis develop bacteremia; of these, 5% to 10% develop localized infections.[157] Bacteremia and metastatic infection are more common among persons with serotypes Choleraesuis, Dublin, Enteritidis, Heidelberg, Poona, and Schwarzengrund and among infants, the elderly, and those with comorbidities, including immunosupression.[158,159,160] Among children, NTS bacteremia usually is associated with gastroenteritis and prolonged fever, infrequently causes focal infections, and is fatal in less than 10% of cases.[161] In contrast, adults are more likely to have primary bacteremia and have a high incidence of secondary focal infections and death.[161] The mortality of NTS bacteremia increases with the duration of bacteremia and in the presence of coma or septic shock.[162,163]

In sub-Saharan Africa, NTS is among the most common causes of bacteremia, and invasive infection is associated with HIV infection in adults and among children with HIV and malaria coinfection, sickle cell disease, or malnutrition.[53] Increasingly, NTS blood isolates from sub-Saharan Africa belong to a single *S.* Typhimurium sequence type, ST313. This strain type has spread rapidly throughout the region and is associated with genome reduction that could make these strains more human adapted with direct human-to-human transmission and more likely to cause a typhoid-like illness than gastroenteritis. However, recent evidence indicates that these strains may be less fit for resistance to oxidative and nitrosative killing, indicating that the long-term colonization of individuals with these strains may have selected for less virulence for those with normal immune responses.[10,164]

NTS endovascular infection should be suspected in cases of high-grade or persistent bacteremia, especially in persons with preexisting valvular heart disease, atherosclerotic vascular disease, prosthetic vascular grafts, or aortic aneurysms (Fig. 223.5). The risk of endovascular infection complicating *Salmonella* bacteremia is estimated to be 9% to 25% in persons older than 50 years, usually involves the aorta, and most commonly results from seeding atherosclerotic plaques or aneurysms.[165,166]

FIG. 223.5 *Salmonella* Enteritidis mycotic pseudoaneurysm of the iliac artery. Contrast computed tomography scan of the abdomen and pelvis (coronary view), showing a pseudoaneurysm of the left common iliac artery *(arrow),* with mass effect on the psoas muscle and left ureter, resulting in significant hydronephrosis *(asterisk). (From Farmakiotis D, Chien KS, Shum TCT, Rodriquez-Barradas M, Musher DM. To scan or not to scan.* Clin Infect Dis*. 2013;56:1003. Used with permission.)*

Using a simple scoring system that assigns +1 point for each of four risk factors (male gender, hypertension, coronary artery disease, and serogroup C1 infection) and −1 point for each of two variables negatively associated with vascular infection (immunsuppression and malignancy), patients with NTS bacteremia can be stratified into high-risk (>1 point, sensitivity 95%, specificity 45%) and low-risk (0–1 point) vascular infection groups.[167] Mortality rates range from 14% to 60% and are lower with prompt diagnosis and combined medical and surgical therapy.[159,161] Endocarditis and arteritis are rare but are associated with potentially fatal complications, including valve perforation, endomyocardial abscess, infected mural thrombus, pericarditis, mycotic aneurysms, aneurysm rupture, aortoenteric fistula, and vertebral osteomyelitis.

Salmonellosis and HIV Infection

NTS is a leading cause of community-acquired bacteremia in HIV-infected adults, especially in sub-Saharan Africa.[168] In developed countries during the pre–antiretroviral therapy (ART) era, NTS bacteremia occurred 20 to 100 times more commonly among those with HIV infection compared with the general population, likely reflecting both increased host susceptibility and impaired clearance.[169] Among HIV-infected patients, NTS bacteremia is associated with lower CD4 lymphocyte counts and a higher risk of metastatic complications, recurrent bacteremia, and mortality despite antimicrobial therapy, especially in Africa.[168]

Recurrent NTS bacteremia is an AIDS-defining illness that apparently results from incomplete clearance of the primary infection because of impaired cell-mediated immunity and dysregulation of proinflammatory cytokines release.[170] In the pre-ART era, as many as 43% of patients with NTS bacteremia had one or more recurrent episodes.[168] Among patients receiving ART, the incidence of recurrent NTS bacteremia has declined up to 96%.[171] The risk reduction is likely due to the impact of ART on virologic suppression and immune reconstitution, a direct bactericidal activity of some antiretrovirals on *Salmonella* spp., and the prophylactic use of trimethoprim-sulfamethoxazole (TMP-SMX).[171]

Localized Infections

Extraintestinal focal infections develop in approximately 5% to 10% of persons with *Salmonella* bacteremia, and their diagnosis and management are summarized in Table 223.2.

Chronic Carrier State

The chronic carrier state is defined as the persistence of *Salmonella* in stool or urine for more than 12 months after acute infection. Chronic carriage of *Salmonella* is associated with excretion of large numbers of organisms in stool but with an absence of clinical disease, related to high levels of systemic immunity. From 0.2% to 0.6% of adults with NTS infection develop chronic carriage, most commonly associated with underlying host immunosuppression.[172] In comparison, up to 10% of untreated patients with typhoid fever excrete *S.* Typhi in the feces for up to 3 months, and 1% to 4% develop chronic carriage, especially associated with biliary abnormalities and gallstone biofilm formation.[173,174]

IMMUNIZATION AGAINST *SALMONELLA*

There is no available vaccine to protect against invasive NTS disease. Two typhoid vaccines currently are commercially available: (1) Ty21a, an oral, live-attenuated *S.* Typhi vaccine (given on days 1, 3, 5, and 7, with a booster every 5 years) and (2) a parenteral Vi capsular polysaccharide vaccine (Vi CPS), consisting of purified Vi polysaccharide from the bacterial capsule (given as a single 0.5-mL [25-µg] intramuscular dose with a booster every 2 years). Typhoid vaccines are reviewed in Chapter 100.

THERAPY

Gastroenteritis

Salmonella gastroenteritis is usually a self-limited disease, and therapy primarily should be directed to the replacement of fluid and electrolyte losses. In a large meta-analysis, antimicrobial therapy for uncomplicated NTS gastroenteritis, including short-course or single-dose regimens with oral fluoroquinolones, amoxicillin, or TMP-SMX, did not significantly decrease the length of illness, including duration of fever or diarrhea, and was associated with an increased risk of positive stool culture 1 month after treatment and adverse drug reactions.[155] Therefore antimicrobials should not be used routinely to treat uncomplicated NTS gastroenteritis or to reduce convalescent stool excretion.

Although less than 5% of all patients with *Salmonella* gastroenteritis develop bacteremia, certain patients are at increased risk for invasive infection and may benefit from preemptive antimicrobial therapy. Antimicrobial therapy should be considered for neonates (probably up to 3 months of age), those older than 50 years with suspected atherosclerosis, and for persons with immunosuppression, cardiac valvular or endovascular abnormalities, or significant joint disease. Treatment should consist of an oral or intravenous (IV) antimicrobial administered for 48 to 72 hours or until the patient becomes afebrile. Immunocompromised persons, including those with HIV infection, who develop *Salmonella* gastroenteritis may require 7 to 14 days of therapy, typically with a fluoroquinolone, to reduce the risk of extraintestinal spread.[175] For susceptible organisms, oral therapy with a fluoroquinolone, TMP-SMX, or amoxicillin is adequate. Although fluoroquinolones are not recommended for administration to children younger than 10 years, they may have a role in treating severe NTS gastroenteritis in this age group, particularly among immunocompromised patients.[176] On occasion, antimicrobial prophylaxis has been required to control institutional outbreaks, especially in long-term care facilities or pediatric wards, where compliance with infection control measures may be difficult.[177]

Bacteremia

Because of the increasing prevalence of antimicrobial resistance, empirical therapy for bacteremia or focal infection suspected to be caused by NTS should include a third-generation cephalosporin and a fluoroquinolone until susceptibilities are known. High-grade bacteremia (i.e., >50% of three or more blood cultures testing positive) should prompt evaluation for endovascular abnormalities by echocardiogram or other imaging techniques, such as computerized tomography or indium-labeled white blood cell scan. Low-grade bacteremia not involving vascular

TABLE 223.2 Extraintestinal Infectious Complications of Salmonellosis

SITE	INCIDENCE	RISK FACTORS	MANIFESTATIONS	COMPLICATIONS	MORTALITY	DIAGNOSIS	THERAPY
Endocarditis	0.2%–0.4%	Preexisting valvular heart disease	Valvular vegetation, infected mural thrombus	Valve perforation, relapse (20%–25%), pericarditis	≈70%	Blood culture, echocardiography	Early valve surgery + 6 wk P ceph 3, P ampicillin, or P, then PO fluoroquinolone
Arteritis	Rare	Atherosclerosis, aortic aneurysm, endocarditis, prosthetic graft, myelodysplasia	Prolonged fever, pain in back, chest, or abdomen	Mycotic aneurysm, aneurysm rupture, aortoenteric fistula, vertebral osteomyelitis	14%–60%	Blood culture, sonogram, MRI or CT	Early surgical intervention + 6 wk P ceph 3, P ampicillin, or P, then PO fluoroquinolone
Central nervous system	0.1%–0.9%	Infants (especially neonates)	Meningitis, ventriculitis, brain abscess, subdural empyema, encephalopathy	Seizures, mental retardation, hydrocephalus, brain infarction, relapse	≈20%–60%	CSF culture, CT or MRI	≥3 wk P ceph 3, P ampicillin, or a carbapenem
Pulmonary	Rare	Lung malignancy, structural lung disease, sickle cell anemia	Pneumonia	Lung abscess, empyema, bronchopleural fistula	≈25%–60%	Respiratory culture, chest radiograph	≥2 wk P/PO abx
Bone	<1%	Sickle cell anemia, male gender, connective tissue disease, immunosuppression	Femur, tibia, humerus, lumbar vertebrae	Relapse, chronic osteomyelitis	Very low	Bone radiograph	≥4 wk P ceph 3, P ampicillin, or P, then PO fluoroquinolone + surgery for sequestra
Joint, reactive	0.6%	HLA-B27, antimicrobial therapy	Joints (≥ three joints) involved (especially knee, ankle, wrist, and sacroiliac)	Prolonged symptoms (mean duration, 5.5 mo)	Negligible	Joint fluid examination and culture	Nonsteroidal antiinflammatory agent
Joint, septic	0.1%–0.2%	Osteoarthritis, connective tissue disease, sickle cell disease, prosthetic joint	Knee, hip, shoulder	Joint destruction, osteomyelitis	Very low	Joint fluid examination and culture	Repeated needle aspiration + ≥4 wk P/PO abx
Muscle/soft tissue	Rare	Local trauma, male gender, diabetes, HIV infection	Abscess, pyomyositis	Osteomyelitis, endovascular infection, frequent relapse	≈33%	Ultrasonography, aspiration	Drainage + ≥2 wk P abx
Hepatobiliary	Rare	Cholelithiasis, cirrhosis, amebic abscess, echinococcal cyst, hepatocellular carcinoma	Hepatomegaly, cholecystitis, hepatic abscess	Rupture with secondary peritonitis, subphrenic abscess, spontaneous bacterial peritonitis	≈10%	Ultrasonography, aspiration	Drainage + ≥2 wk P abx
Splenic	Rare	Sickle cell anemia, splenic cyst, splenic hematoma	Splenomegaly	Left pleural empyema, subphrenic abscess, rupture with secondary peritonitis	<10%	Ultrasonography, aspiration	≥2 wk P abx + percutaneous drainage or splenectomy
Urinary	0.6%	Urolithiasis, malignancy, renal transplant, elderly female	Cystitis, pyelonephritis	Renal abscess, interstitial nephritis, relapse	≈20%	Urine culture, ultrasonography	Removal of structural abnormality + 1–2 wk P abx + ≥6 wk PO fluoroquinolone or TMP-SMX
Genital	Rare	Pregnancy, renal transplant	Ovarian abscess, testicular abscess, prostatitis, epididymitis	Abscess	Very low	Ultrasonography, aspiration	Drainage of collection + 1–2 wk P abx + ≥6 wk PO fluoroquinolone or TMP-SMX
Soft tissue	<1%	Local trauma, immunosuppression	Pustular dermatitis, SC abscess, wound infection	Septic thrombophlebitis, endophthalmitis	≈15%	Drainage culture	≥2 wk P abx + drainage of collection

abx, Antibiotics; *CSF,* cerebrospinal fluid; *CT,* computed tomography; *HIV,* human immunodeficiency virus; *HLA,* human leukocyte antigen; *MRI,* magnetic resonance imaging, *P abx,* parenteral ampicillin; *P ceph 3,* parenteral third-generation cephalosporin; *PO,* oral; *P/PO abx,* parenteral or oral antibiotic (e.g., fluoroquinolone, ampicillin, TMP-SMX, or P ceph 3); *P/PO fluoroquinolone,* parenteral or oral fluoroquinolone; *SC,* subcutaneous; *TMP-SMX,* trimethoprim-sulfamethoxazole.

structures should be treated with 7 to 14 days of IV ceftriaxone or ampicillin or by IV, followed by oral, fluoroquinolone therapy. Documented or suspected endovascular infection should be treated with 6 weeks of IV ceftriaxone or IV ampicillin or by fluoroquinolone therapy (initially administered IV followed by oral when stable). Early surgical resection of infected aneurysms or other endovascular focus on infection is recommended. Alternatively, patients who are not candidates for surgical resection and vascular grafting have been treated with endovascular stenting and/or chronic suppressive oral antimicrobial therapy.[178]

Recurrent *Salmonella* Bacteremia in Persons With HIV

For HIV-infected persons with CD4 counts ≥200 cells/mm^3, a first episode of NTS bacteremia can be treated with 14 days of ceftriaxone or ampicillin or by IV, followed by oral fluoroquinolone therapy, provided that the bacteremia rapidly clears. For those persons with persistent bacteremia, metastatic complications, or CD4 count <200 cells/mm^3, 4 to 6 weeks of therapy is recommended.[23] Persons who relapse after 4 to 6 weeks of antimicrobial therapy should receive long-term suppressive therapy with an oral fluoroquinolone or TMP-SMX, based on susceptibility testing.

Focal Infections

Treatment recommendations for the management of focal *Salmonella* infections are summarized in Table 223.2. For extraintestinal nonvascular infections, antimicrobial therapy for 2 to 4 weeks (depending on the infection site) is usually recommended. In cases of chronic osteomyelitis, abscesses, and urinary or hepatobiliary infection associated with anatomic abnormalities, surgical resection or drainage may be required in addition to prolonged antimicrobial therapy to eradicate infection.

Carrier State

Treatment of persons with asymptomatic carriage of NTS is controversial. In a randomized, placebo-controlled trial conducted in Thailand among asymptomatic food workers, two 5-day regimens (norfloxacin, 400 mg twice daily, and azithromycin, 500 mg once daily) were no more effective than placebo in eradicating NTS carriage and increased the risk of reinfection with antimicrobial-resistant *S.* Schwarzengrund.[179]

Prevention and Control

The prevention and control of NTS require both an understanding of the complex cycles of transmission and ongoing surveillance to characterize trends in *Salmonella* incidence, prevalence, and antimicrobial susceptibility and to identify outbreaks. In developing countries, especially in sub-Saharan Africa, controlling the high burden of invasive NTS will require enhanced surveillance to estimate disease burden, rapid diagnostics to identify antimicrobial resistance, and clinical studies and management algorithms to improve patient outcomes.

In developed countries, control of foodborne salmonellosis requires identification of controllable hazards, monitoring, and verification to limit the introduction and multiplication of *Salmonella* from the farm to the table.[180] Recognition of foodborne outbreaks requires that clinicians have a high index of suspicion, order the appropriate laboratory test, and promptly report positive culture results to local public health departments. Vaccination of livestock and egg-laying hens, further limiting the use of antimicrobials as animal growth promoters, and improved food safety practices should further reduce the burden of foodborne salmonellosis. Active population-based surveillance for foodborne diseases has improved estimates of disease burden,[4] and use of algorithms and rapid molecular subtyping has improved the ability to detect and more rapidly control outbreaks of salmonellosis associated with widely distributed agricultural and manufactured foods.[181] Although most cases of *Salmonella* infection occur sporadically, large numbers of persons potentially may become infected when commercial kitchens serve *Salmonella*-contaminated foods that have not been sufficiently cooked or that have been mishandled. Commercial food service establishments can reduce the risk of foodborne *Salmonella* illness if they do not serve food containing raw or undercooked eggs, use pasteurized eggs whenever possible, and avoid cross-contamination of food items. Use of pasteurized eggs for all recipes calling for bulk-pooled eggs is recommended for all nursing homes and hospitals.

The most cost-effective approach to the control of salmonellosis in food handlers is attention to good personal hygiene and maintenance of time-temperature standards for food handling. Routine screening of food handlers for carriage after gastroenteritis is commonly performed before they are allowed to return to work. However, there is little justification for this approach because few outbreaks are related to specific food handlers, prolonged carriage in food handlers after gastroenteritis is rare, and the number of organisms present is small. Therefore it is reasonable to allow individuals to return to work after diarrhea is resolved. Two consecutive negative stool samples should be required only for food handlers whose work involves touching unwrapped foods that are consumed raw or served without further cooking. Routine surveillance of food handlers for asymptomatic stool carriage of *Salmonella* is not recommended.

To limit the risk of health care–associated transmission to patients and personnel, patients excreting NTS should be managed with standard precautions, including appropriate hand hygiene and the use of personal protective equipment, including gloves, when performing direct patient care or handling fecally soiled articles. Additional measures, including enhanced environmental disinfection and cohorting of ill patients may be required to control outbreaks of NTS infection in health care facilities.[182] Although NTS infection in newborns, the elderly, or the immunocompromised can be severe, the risk of transmission of *Salmonella* from health care workers to patients appears to be very small.[63] Once the health care worker is asymptomatic and passing formed stool, the individual should be allowed to return to work if standard precautions are observed. However, local and state regulations should be followed because some require work exclusion for health care workers who have salmonellosis until two or more stool cultures obtained at least 24 hours apart are negative.

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The complete reference list is available online at Expert Consult.

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224 Bacillary Dysentery: *Shigella* and Enteroinvasive *Escherichia coli*

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SHORT VIEW SUMMARY

Definition
- Diarrheal illness caused by strains of invasive forms of *Shigella* or invasive *Escherichia coli* comprises bacillary dysentery.

Epidemiology
- Being a very low-inoculum disease, shigellosis is the most infectious (communicable from person to person) of the bacterial enteropathogens.

Microbiology
- *Shigella* spp. are small gram-negative rods in the family Enterobacteriaceae.
- Similar to strains of *Shigella,* enteroinvasive *E. coli* (EIEC) possesses somatic antigens and a plasmid that controls invasiveness.

Diagnosis
- Patients passing bloody stools or those associated with cases of shigellosis should have stools processed for *Shigella.*
- The sooner a stool specimen is cultured, the higher the yield for *Shigella.*
- Once specimens have been plated on gram-negative media at 37°C and incubated overnight, lactose-negative colonies should be tested biochemically and serologically for *Shigella.*
- Nucleic acid amplification tests (NAATs) improve the sensitivity for the diagnosis of dysentery but target a gene shared by both *Shigella* and EIEC, and therefore the report does not distinguish between the two organisms. Furthermore, NAATs do not provide information on species or drug susceptibility.
- *Shigella* or EIEC identified through molecular methods should be confirmed by culture for speciation, serotyping, and drug susceptibility testing and for epidemiologic purposes.

Therapy
- Antibiotics are useful in the management of shigellosis and can be lifesaving for infection caused by the Shiga bacillus (*Shigella dysenteriae* type 1).
- The treatment of choice for adults is a fluoroquinolone antibiotic given for 3 days.
- For children, cephalosporin, ciprofloxacin, or azithromycin may be used.
- Antimicrobial resistance is an emerging problem worldwide, with increasing reports of strains that are resistant to fluoroquinolones, macrolides, and cephalosporins.

Prevention
- Because person-to-person spread is so important in *Shigella* infection, hand washing and other hygienic methods should be used to prevent spread.
- Vaccines are in development to prevent *Shigella* infection.

The term *dysentery* was used by Hippocrates to indicate a condition characterized by the frequent passage of stool containing blood and mucus, accompanied by straining and painful defecation. It was not until the end of the 19th century, when the causes of amebiasis and bacillary dysentery were determined, that the two great forms of dysentery could be accurately separated. In view of the absence of liver complications, much of the dysentery in the older historical writings is considered to be of bacillary origin (shigellosis). After the causative agents of the two types of dysentery were determined, the different epidemiologic settings were described. In 1859 in Prague, Lambl and then later Osler[1] and Councilman and Lafleur[2] helped verify the pathogenicity of *Entamoeba histolytica.* In 1906, Shiga conclusively demonstrated that a bacterium was present in the stool of many patients with dysentery and that agglutinins could be demonstrated in the serum of the infected patients.[3] At about the same time, Flexner found a similar but serologically different organism in the stools of other patients with dysentery acquired in the Philippines.[4] Rogers stated in 1913 that "epidemic dysentery in asylums, jails, or in long-occupied and unsanitary military camps during the war is almost certain to be bacillary, while sporadic cases in a warm climate are more frequently amebic."[5]

Medical writings since the beginning of recorded history have dealt with the common problems of dysentery in civilian and military populations; perhaps the greatest historical consideration is the influence that bacillary dysentery has had on military campaigns. Almost every long campaign and extended siege has produced epidemics of bacillary dysentery, particularly when sanitation and food sources could not be adequately controlled. In many military battles leading up to World War I, a heavier toll was ascribed to bacillary dysentery than to war-related injuries.[6]

MICROBIOLOGY

Shigella organisms are small gram-negative rods that are members of the family Enterobacteriaceae, tribe Escherichieae, and genus *Shigella.* They are nonmotile and nonencapsulated. Comparative genomics using whole-genome sequencing has suggested that *Shigella* and enteroinvasive *Escherichia coli* (EIEC) include diverse members with virulence mechanisms in common acquired through mobile genetic elements derived from plasmids, phage, or genomic islands. *Shigella* evolved by acquiring virulence genes from multiple lineages of *E. coli*[7]; more recently, EIEC lineages evolved independently from multiple distinct lineages of *E. coli* via the acquisition of *Shigella* virulence plasmid and in some cases *Shigella* pathogenicity islands.[8]

Shigellae are polyphyletic, meaning that they are not all descendants of a single clone. One hypothesis is that members of several *E. coli* clones acquired a mobile genetic element—the ancestor of the present-day invasion plasmid.[9] Through adaptation to the plasmid and additional convergent evolution with the acquisition of mobile genetic elements, deletions or mutations affecting gene function lead to the loss of motility and other phenotypes such as intracellular niche and the ability to spread from cell to cell that distinguish *Shigella* from *E. coli,*[10] This apparently happened multiple independent times, resulting in the various *Shigella* lineages. However, evolutionary reconstructions place *Shigella* squarely within the broad range of lineages that we call *E. coli.*[10]

Isolation Techniques

The infecting strain of *Shigella* is generally present in stool in concentrations between 10^3 and 10^9 viable cells per gram of stool, depending on the stage of illness. During the postconvalescent shedding period, counts fall to 10^2 to 10^3 viable cells per gram of stool. Recovery of the agent microbiologically is not usually difficult in the early stages of disease because of the higher counts present; it is more difficult during later stages of illness because of the lower counts of viable bacteria. Patients with shigellosis at the height of their illness can have negative stool cultures.[11] Careful selection of feces and processing on appropriate media give a higher yield of organisms. The sooner after passage the specimen is processed, the

higher is the yield. Stool that stands at room temperature for more than 24 hours has a profound drop in the number of viable cells, and recovery of the pathogen is less likely. A rectal swab obtained and seeded immediately at the bedside is the optimal way to perform a stool culture.

For bacteriologic identification of *Shigella*, a bit of blood or mucus is seeded onto at least two different media. In general, stool is plated lightly onto a medium with only mild inhibiting factors for gram-negative growth, such as MacConkey agar, xylose-lysine-deoxycholate agar, Tergitol 7, or eosin–methylene blue (EMB) agar, whereas a separate specimen is plated heavily onto a more inhibitory medium, such as *Shigella-Salmonella* medium. The more plates used, the greater the recovery yield. After overnight incubation at 37°C, lactose-negative colonies are tested biochemically and then serologically identified with *Shigella* grouping and typing antisera.

Group and Type Identification

Shigella are divided into four groups, depending on serologic similarity and fermentation reactions: group A *(Shigella dysenteriae),* with 15 serotypes; group B *(Shigella flexneri),* with 19 serotypes and subserotypes; group C *(Shigella boydii),* with 20 serotypes; and group D *(Shigella sonnei),* with a single serotype. Commercial antiserum is available for determining group- and type-specific antigenicity. *S. sonnei* accounts for 60% to 80% of the cases currently reported in the United States and other industrialized areas.

Invasive *Escherichia coli*

Certain strains of *E. coli* can cause a clinical illness indistinguishable from shigellosis and should be considered as causative agents of bacillary dysentery. Almost all the *Shigella*-like *E. coli* strains have been shown to possess somatic antigens related to *Shigella* serotypes, further demonstrating the similarity of these two groups of organisms. EIEC strains that cause bacillary dysentery have been shown to belong serologically to the following *E. coli* O groups: 28, 29, 112, 115, 124, 136, 143, 144, 147, 152, 164, and 167. Serotyping may ultimately prove to be useful in detecting these strains. At the genomic level, most EIEC strains are distributed across three lineages.[8] The classic laboratory test for determining the virulence of a bacterial isolate (*Shigella* or EIEC strain) was the Sereny test.[12] Keratoconjunctivitis develops after 1 to 7 days in guinea pigs (or rabbits) when an invasive bacterial strain (*E. coli* or *Shigella*) is dropped into the conjunctival sac of the animal (Fig. 224.1). This test is no longer used for diagnostic purposes. As discussed earlier, *Shigella* and EIEC have evolved from common ancestors and conceptually form a single pathovar within *E. coli,* with different lineages.[13]

A different form of bacillary dysentery has been shown to be caused by an O157:H7 strain of *E. coli* and other Shiga toxin (STx)–producing *E. coli* strains (see Chapter 218).

PATHOGENESIS

Communicability and Infectivity

Shigellosis is *the* most communicable of the bacterial diarrheas. Experiments in volunteers have demonstrated that shigellosis is unique among bacterial enteropathogens in that fewer than 100 viable cells can readily produce the disease in healthy adults.[14] Dose-response data obtained in volunteers for virulent strains from three species of *Shigella* are given in Table 224.1. When volunteers ingested 500 or fewer viable cells of *S. flexneri, S. sonnei,* or *S. dysenteriae* type 1 (the Shiga bacillus), essentially the same rate of clinical illness resulted—27% to 45%.[14] In general, the laboratory-passaged strains used in volunteer studies are less infectious than naturally transmitted strains, implying that the actual infectious dose in the field is considerably lower than 500 organisms. This low dose of organisms probably explains how the illness can be so readily transferred from person to person, why the secondary attack rate is so high when an index case is introduced into a family, and why recurrent bacillary dysentery is an important problem in institutionalized or crowded populations.

The reasons for this low dose response are not completely clear. One possible explanation is that virulent shigellae can withstand the low pH of gastric juice. In a study of adult Bangladeshi men admitted to the hospital with diarrhea, normal gastric acid levels were seen in patients with shigellosis, amebiasis, and pathogen-negative diarrhea, whereas patients with secretory diarrhea caused by *Vibrio cholerae* and enterotoxigenic *E. coli* had low gastric acid levels, offering evidence that *Shigella* did not require reduced gastric acidity to produce enteric disease.[15] In another study, *Shigella* isolates were able to survive at a pH of 2.5 for at least 2 hours, whereas *Salmonella* was not.[16] *Shigella* strains were also shown to be able to survive in acidic apple juice and tomato juice stored at 7°C and 22°C, respectively, for up to 14 days, showing its resistance to acid.[17] A study in Kenyan children suggested that unabsorbed iron could be a direct risk for pathogens or for intestinal dysbiosis.[18] Although EIEC and *Shigella* possess the same virulence determinates, infection with IEC requires an average dose 1000 times higher to cause infection.[19] EIEC strains have not been compared with *Shigella* to determine if relative acid susceptibility might explain the different dose response. Nonpathogenic *E. coli* strains appear to have similar acid susceptibility to strains of *Shigella,* suggesting that this is not the reason for the difference in dose response.[16]

Mucosal Invasion and Inflammation

Virulent *Shigella* and other nontoxigenic invasive *E. coli* strains produce disease after invading the intestinal mucosa via the basolateral surface of the colonic enterocyte.[20,21] Genes required for bacterial entry into epithelial cells are present on a 30-kb region of a 220-kb virulence plasmid.[22] *Shigella* infection is superficial, and only rarely does the organism penetrate beyond the mucosa, which explains the rarity of obtaining positive blood cultures in patients with shigellosis, despite the common occurrence of hyperpyrexia and toxemia. *Shigella* and EIEC invade colonic and rectal cells, including M cells of the follicle-associated epithelium, macrophages, and epithelial cells; invasion is followed by intracellular multiplication, spread of infection to adjacent cells, severe inflammation, and destruction of colonic mucosa.[23] Apoptotic destruction of macrophages in subepithelial tissue allows survival of

FIG. 224.1 Guinea pig with keratoconjunctivitis after conjunctival inoculation of invasive *Escherichia coli*. This is a positive Sereny test result but is no longer used for diagnosis.

TABLE 224.1 Response of Adult Volunteers to Experimental Challenge With Viable Virulent Strains of *Shigella*

SHIGELLA SPECIES	INOCULUM (ORGANISMS)	NO. OF VOLUNTEERS	NO. OF CASES OF CLINICAL SHIGELLOSIS (% OF TOTAL)
S. flexneri (strain 2467T)	≤180 ≥5×10^3	72 211	23 (32) 124 (59)
S. sonnei (53G)	500	58	26 (45)
S. dysenteriae 1[a]	≤200 ≥2×10^3	22 22	6 (27) 14 (64)

[a]Strains A-1 and M-131.

Modified from DuPont HL, Levine MM, Hornick RB, et al. Inoculum size in shigellosis and implications for expected mode of transmission. J Infect Dis. *1989;159:1126–1128.*

the invading shigellae, and inflammation facilitates further bacterial entry.[24] Once the organisms are intracellular, they multiply within the cytoplasm and move from cell to cell by an actin-dependent process.[23]

Pathogenic strains of *Shigella* and other bacterial enteropathogens have evolved a complex type III secretion mechanism that enables them to invade the intestinal mucosa.[25] Bacterial proteins (including toxins) are injected from the bacterial cytoplasm into the cytosol of host mucosal cells, where they modulate the functions of the host cells and dictate how the host and pathogen relate.[26–28] Each type III system consists of the secretion apparatus, secreted effector proteins, cytoplasmic chaperones (specialized for transporting the specific effector proteins), and specific transcriptional regulators.[29–31] The secreted proteins—there are approximately 20, including VirA, OspB to OspG, IpaA to IpaD, and IpgD—facilitate bacterial entry into nonphagocytic cells and induce apoptosis and are targets for vaccine development.[28] *Shigella* strains decrease production of host antimicrobial peptides, thus facilitating persistence by avoiding the host immune system.[32] Strains of bacterial pathogens that use type III secretion systems can be detected by screening for virulence genes directly.[33]

Toxigenicity

All four species of *Shigella* can produce the plasmid-borne toxin ShET2,[34] whereas the chromosomally encoded ShET1 is elaborated by *S. flexneri* 2a,[35] and STx is produced by *S. dysenteriae* 1.[36] These toxins can induce water and electrolyte secretion across the colonic epithelium. However, other enterotoxins are likely involved in secretory diarrhea because nontoxigenic strains can also cause disease. STx is similar to the STx1 and STx2 produced by enterohemorrhagic *E. coli* O157:H7 and other Shiga toxin–producing *E. coli* (STEC) strains. A high proportion of children with *S. dysenteriae* 1 develop STx-related hemolytic uremic syndrome.[37]

Anatomic Location of Infection

Studies in volunteers have helped establish the intestinal localization of bacteria in experimental shigellosis.[11] Within 12 hours after participants swallow virulent shigellae, the bacteria transiently multiply in the small bowel to concentrations of 10^7 to 10^9 viable cells/mL of luminal contents, at which time abdominal pain, cramping, and fever occur. Within a few days, the infecting strain is no longer detectable in small bowel fluid, the patient's temperature becomes lower, and pain and tenderness, generally confined to the lower abdominal quadrants, become more severe. Urgency, tenesmus, and passage of bloody mucoid stools (dysentery) often occur in the later stages of infection and correlate with a diffuse colonic localization of the bacteria. Although strains of *Shigella* appear to be resistant to acid, as discussed earlier, acid exposure may transiently inhibit the virulence properties of the organism, which may encourage transit through the small bowel to the colon, where virulence characteristics are once more produced.[38] The density of intramucosal bacteria is highest at the luminal surface and extends in decreasing concentrations to reach the lamina propria and submucosa. Microabscesses form and coalesce, becoming large abscesses that slough and produce mucosal ulcerations. In shigellosis, both humoral and cellular immune mechanisms are stimulated. Cytokine levels correlate with disease severity,[39] and a number of fecal cytokines, including interleukin (IL)-8 and IL-1β, are higher than seen with other enteric bacterial pathogens.[40]

EPIDEMIOLOGY

Hippocrates indicated that when a dry winter was followed by a rainy spring, an increase in the number of dysentery cases would follow in the summer. In general, bacillary dysentery is a summertime illness, where it is characteristically seen in children living in crowded areas with inadequate sanitation and limited water. Because of the characteristic clinical picture of bacillary dysentery, it is one of the most accurately diagnosed and reported classes of infectious diarrhea. The greatest frequency of illness is reported in infants and younger or preschool children. Disease rates and also complications and severity parallel the degree of malnutrition. Flies may be important in the transmission of bacillary dysentery,[41,42] especially in tropical climates. Dysentery in warm countries is most prevalent when the fly population is at its highest. Bacteriologic surveys of fly populations have indicated that flies can occasionally be shown to be positive for *Shigella* bacteria.[41] The low dose required for infection at least partially explains the potential for fly transmission of shigellosis. Fly control, hand washing, and breast-feeding show protective effects against the organism.[43]

Cyclic Patterns of Disease

Since the description of bacteriologic isolation procedures, cyclic epidemics of bacillary dysentery have been described, each cycle lasting 20 to 50 years.[44] In Europe, during the first 25 years of the 20th century, dysentery was generally caused by *S. dysenteriae* 1, and mortality was higher than subsequently seen when other serotypes became prevalent. Between 1926 and 1938, *S. flexneri* strains became more prevalent than the Shiga bacillus in the developing world, and *S. flexneri* remains the major *Shigella* type in these areas. *S. sonnei* has become the major cause of bacillary dysentery in European countries and the United States. Widespread epidemics in the developing world may be seen for the more virulent *S. dysenteriae* 1, resulting in deaths without proper antimicrobial therapy. In general, *S. flexneri* and *S. sonnei* represent the endemic *Shigella* species in most populations, but in situations in which hygienic standards are lower, *S. dysenteriae* 1 can cause epidemic disease. Epidemics may occur in 20- to 30-year cycles, believed to reflect the development of immunity followed by the birth every year of more and more susceptible individuals until the conditions are ripe for epidemic transmission. Shiga dysentery remains a special problem in parts of Africa, the Indian subcontinent, and Bangladesh, where epidemic strains showing greater resistance to phagocytosis cause pandemic disease.[45]

Incidence of Shigellosis by Geography and Host

The annual number of *Shigella* episodes worldwide has decreased from 80 to 165 million cases and 600,000 deaths annually in the 1990s[46] to an estimated 270,00 deaths in 2016.[47] The highest rate of *Shigella* infection (69% of cases) and the highest death rate (61% of deaths) occur in those younger than 5 years. From 2006 to 2015, between 1190 and 2308 culture-confirmed cases were reported annually in the United Kingdom.[48] In the United States 2132 cases were reported to the CDC in 2017. Of these, 31% were diagnosed with nucleic acid amplification tests (NAATs).[49] Considering that not all cases are studied, the actual number of cases may reach 500,000 in the United States.

In numerous published studies, a causative agent has been identified in 10% to 50% of pediatric diarrhea cases, depending on geographic location, severity of illness, and laboratory methods used.[50–52] Bacillary dysentery is primarily a disease of children 6 months to 10 years of age, although adults often acquire the illness from their children, or acquire it when traveling to developing countries. Bacillary dysentery does not commonly develop in children younger than 6 months but is the most common prevalent pathogen in children 24 to 59 months old in sub-Saharan Africa.[53] However, in industrialized countries, *Shigella* strains may rarely cause severe illness in newborns,[54] but in developing countries, where breastfeeding is more common, infants are resistant to shigellosis,[55] probably because of exclusion from contaminated food or drink, changes in the intestinal microbiota of breastfed children, or the presence of specific antibody in breast milk. Shigellosis has become an important problem in daycare centers for preschool children in the United States, and in religious communities with large family size.

Modes of Spread and Reservoirs in Nature

Many cases of bacillary dysentery in industrialized regions are a result of person-to-person transmission. Widespread epidemics have occurred in military or civilian populations and among persons who have ingested contaminated food or water on cruise ships. Water and food appear to be particularly important vectors of *Shigella* transmission in developing countries, where they may be the most important sources of infection.[56,57] Epidemics of waterborne shigellosis generally appear to be the result of wells contaminated with fecal material. Felson[58] found that dysentery strains could be recovered for up to 6 months from water samples maintained at room temperature. Wells are often located close to cesspools

and outhouses in developing countries, where sanitation principles are not followed. In other areas, septic tank discharge may empty into lakes, ponds, or other bodies of water close to intake lines for camp water supplies or adjacent to bathing beaches. Chlorination of water, if appropriately maintained, will remove the threat of such infections. In the United States, foodborne[59] and waterborne[60] outbreaks of shigellosis occur occasionally and represent 7% of reported cases.[47]

An epidemiologic observation has been made that when water sanitation improvements are implemented in a community, the incidence of typhoid fever falls but the prevalence of bacillary dysentery remains unchanged.[58] *S. sonnei*, the most common species in Western countries, appears to have descended from a common ancestor (monophyletic) existing hundreds of years ago that diversified into distinct lineages in Europe, followed by worldwide dissemination.[61]

Hand transmission is likely to be a common means of acquiring infection. At a custodial institution, intellectually disabled persons were studied for the prevalence of hand transmission of bacteria.[41] Finger and simultaneous fecal cultures were obtained from 268 institutionalized patients. A *Shigella* strain was isolated from the stool of 39 persons, and the fingers were positive in 4 (10% of those with a positive stool culture). In addition, fecal cultures were found to be negative in an additional 229 patients, whereas a *Shigella* strain was isolated from the hands and fingers of 2 of these patients with negative stool cultures. *E. coli* was recovered from the fingers of 82% of those studied, which demonstrates the common occurrence of fecal organisms on the hands of institutionalized persons. These institutionalized patients had adequate washroom and showering facilities and did not show evidence of decreased personal hygiene.

Outbreaks of shigellosis have been described in men who have sex with men, in some instances due to antimicrobial resistant strains[62] and spreading across several continents.[63]

Secondary transmission of *Shigella* infection is common in households, with rates influenced by the ages of family members.[41,64] After a bout of shigellosis without antimicrobial therapy, fecal excretion of the infecting strain generally lasts 1 to 4 weeks. Long-term *Shigella* carriage rarely occurs. In contrast to typhoid and cholera carriers, in whom the gallbladder or small bowel may be a site of infection, the organisms in dysentery carriage are confined to a colonic site. In the absence of coexistent parasitic infestation of the intestine, these carriers generally respond to antimicrobial therapy. The number of organisms excreted by these persons is generally less than that seen in acute dysentery, and thus the infection in such individuals is less communicable than that in active cases.

DIAGNOSIS

History

Bacillary dysentery should be considered in any patient with acute diarrheal illness associated with toxemia and systemic symptoms, particularly when the illness lasts longer than 48 hours, and when intrafamily spread occurs with an interval of 1 to 3 days between cases, fever is present, or blood or mucus is seen in stool. The occurrence of hyperpyrexia and seizures in infants and children with shigellosis has led some to the conclusion that a neurotoxin is important in the pathogenesis of clinical illness, although there is little to support this notion. In patients able to give a careful history, a descending intestinal tract infection is often described. The first symptoms may be fever and abdominal cramping, followed by voluminous watery stool during small bowel infection, followed by a decrease in fever and an increase in the number of stools passed with smaller volume ("fractional stools") as the colon becomes the site of infection. At that time, the passage of bloody mucoid stools with fecal urgency and tenesmus may develop. Abdominal pain and diarrhea occur in almost all patients with shigellosis, fever can be documented in approximately one-third of cases, and mucus is seen in the stools of 50% and gross blood in 40% of cases.[11]

Physical Examination

Findings of physical examination are nonspecific and include a variable degree of systemic toxemia, fever (which may be as high as 105°F), abdominal tenderness (especially over the lower abdominal quadrants), and hyperactive bowel sounds. Rectal examination or proctoscopy is generally painful, and an abnormally friable hyperemic rectal mucosa, increased mucus secretion, and areas of ecchymosis are generally found. Ulcerations of rectal mucosa can be seen after several days of illness. Rectal prolapse may occur with profuse stooling.

Laboratory Findings

During the acute illness, the infecting strain is present in large enough numbers that stool cultures are generally positive. In the later stages of the disease, it may be necessary first to culture material in enrichment broth before plating. Culture of colonic or rectal biopsy does not improve the efficiency of stool culture in shigellosis.[65] The key to establishing the diagnosis of shigellosis is isolation of the organism from diarrheal stool. Laboratory identification of *Shigella* was discussed earlier (see "Isolation Techniques"). In research centers where the service is available, direct fluorescent antibody microscopy may be useful in detecting the organism when present in small numbers,[66] but because of the numerous serotypes potentially responsible for the infection, this procedure does not have widespread application. Multiplexed NAATs are becoming increasingly popular for the identification of enteropathogens in stool specimens owing to their high sensitivity, rapid turnaround times, and decreased overall costs.[67] Several commercially available platforms detect *Shigella* with a high degree of sensitivity (95.9%) and specificity (99.9%) when stool culture is used as the gold standard.[68] NAATs target a gene shared by both *Shigella* and EIEC, and therefore the report does not distinguish between the two organisms. Furthermore, NAATs do not provide information on species or drug susceptibility. Thus, *Shigella* or EIEC identified with NAATs, should be confirmed by culture for speciation, serotyping, and drug susceptibility testing and for epidemiologic purposes.

The total white blood cell count demonstrates no consistent findings, although leukopenia and brisk leukocytosis are seen on occasion. A shift to the left (an increased number of band cells in comparison to segmented neutrophils) when a leukocyte differential count is performed in a patient with diarrhea suggests bacillary dysentery. The single most important laboratory test, other than stool culture, is direct microscopic examination of a stained fecal smear, which will show numerous polymorphonuclear leukocytes.[69] A wet mount preparation is made by adding stool (mucus, if present) to an equal amount of methylene blue dye. The preparation is then covered with a coverslip and examined microscopically under the high dry objective. Alternatively, the specimen can be heat fixed before staining with dilute methylene blue and examined under an oil immersion objective after drying. Numerous sheets of polymorphonuclear leukocytes are normally found in shigellosis and EIEC diarrhea (Fig. 224.2). Fecal leukocytes indicate a diffuse colitis or proctitis seen in salmonellosis, *Campylobacter* diarrhea, STx–producing invasive *E. coli* colitis, and idiopathic ulcerative colitis. Fecal lactoferrin represents a more sensitive test of mucosal inflammation than a fecal leukocyte test, and is strongly positive in most cases of shigellosis.[70]

Serologic evaluation of a patient with bacillary dysentery is not generally helpful in establishing the diagnosis because humoral antibodies do not develop before recovery. Serologic procedures are helpful as an epidemiologic tool in defining the extent of an epidemic in a population known to be infected by a known *Shigella* serotype (especially the Shiga bacillus). The humoral antibody response correlates with the severity of clinical disease.[11]

Therapy and Clinical Course

In certain patients with bacillary dysentery (particularly in infants and older adults), significant dehydration may result from excessive fluid loss through diarrhea and vomiting. The fluid losses can generally be replaced by oral intake because the diarrhea associated with bacillary dysentery is not normally associated with profound fluid and electrolyte depletion. If vomiting or extreme toxemia is a prominent feature of the illness, especially in the very young or very old, intravenous fluid replacement may be necessary. As in all diarrheal illnesses, fluid repletion is the mainstay of therapy and should be given even as antimicrobial therapies are being considered.

Antibiotics are useful in the management of shigellosis and may be lifesaving in the case of Shiga dysentery. Because the infection is normally self-limited and because antibiotic resistance commonly develops in

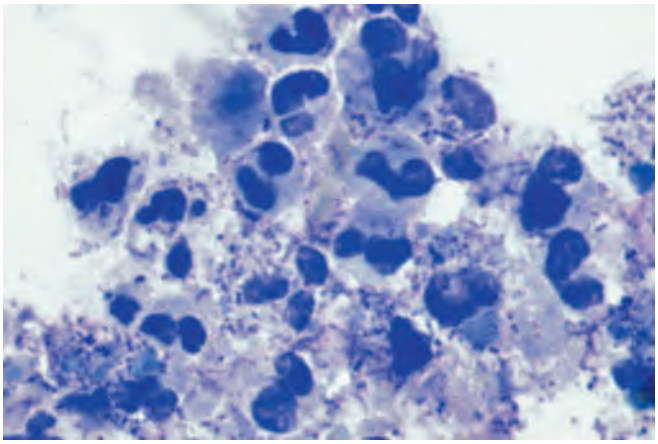

FIG. 224.2 Fecal leukocytes taken from patient with diffuse colitis (methylene blue stain). This exudative response may be seen in shigellosis, salmonellosis, *Campylobacter* infection, and colitis caused by invasive or Shiga toxin–producing *Escherichia coli.*

populations after prolonged use of drugs, some experts and the Centers for Disease Control and Prevention (CDC) recommend that antimicrobial therapy be reserved for the most severely ill patients—those with immunosuppression, with bacteremia, or with complications and those who are hospitalized; for food handlers, residents of nursing homes, or childcare providers; or for situations in which spreading and outbreaks are possible.[71,72] However, because the infection is generally transmitted from person to person and the infected or colonized person represents the major reservoir of infection, for public health reasons individuals with a positive stool culture or with known bacillary dysentery who are living or working in facilities with high risk for transmission (daycare centers, nursing homes) should be treated.

Because of the emergence of drug resistance, the efficacy of fluoroquinolones, trimethoprim-sulfamethoxazole, and macrolides in adults as empirical therapy when susceptibility is unknown has diminished. Risk factors for antimicrobial resistance include foreign travel, particularly to Southeast Asia and Africa; men who have sex with men; and HIV coinfection. For persons without these risk factors, a fluoroquinolone remains the drug of choice; for persons with these risk factors, cefixime or ceftriaxone is a reasonable alternative. For cases in which susceptibility is known, the specific drugs and dosages are indicated in Table 224.2. Trimethoprim-sulfamethoxazole had been the treatment of choice for this enteric infection, but resistance has become widespread for strains of *Shigella.*[73,74,75,76] Although 3-day therapy is generally recommended in shigellosis, single-dose fluoroquinolones may be given for milder forms of shigellosis.[77] For children, various drugs may be used. Cephalosporins have become a common form of treatment of pediatric shigellosis.[78–80] Although not approved for use in children, short-course fluoroquinolones can be safely used.[81] Amdinocillin, an unlicensed drug, has been used in Bangladesh for shigellosis.[81] Azithromycin has been used successfully for treatment of multidrug-resistant *Shigella* infection in adults[82,83] and should be useful in the management of pediatric shigellosis. Nalidixic acid may be helpful in the management of pediatric shigellosis.[84] Rifaximin should not be used to treat shigellosis. However, it has been proven to prevent shigellosis in experimental challenge studies[85] and is an option to prevent traveler's diarrhea, probably by eradicating *Shigella* infection before the organisms reach the colon and establish infection.

Intestinal motility patterns may be important in recovery from infection, and in preventing mucosal invasion by a bacterial agent.[86] In such cases, diarrhea might be viewed as a protective mechanism, and its inhibition by motility-active drugs may be counterproductive. Paregoric has occasionally been shown to worsen clinical salmonellosis[86] and, in occasional patients, antidiarrheal drugs such as diphenoxylate (Lomotil) worsen bacillary dysentery and could play a role in the development of toxic dilatation of the colon or perforation.[87] In dysenteric diarrhea, the antimotility drugs may be safely given if effective antimicrobial drugs are also administered.[88]

TABLE 224.2 Antibacterial Therapy for Patients With Shigellosis

ADULTS		CHILDREN	
Agent	**Dosage**	**Agent**	**Dosage**
Ceftriaxone	1 g IV once daily for 3–5 days	Ceftriaxone	50 mg/kg IV once daily (maximum, 2 g/day) × 5 days
Cefixime	200 mg orally twice daily for 5 days		
Ciprofloxacin[a]	500 mg bid × 3 days	Ciprofloxacin[b]	25 mg/kg/day, divided q12h × 3–5 days (daily adult dose 1–1.5 g/day)
Levofloxacin[a]	500 mg daily × 3 days		
Azithromycin	500 mg qd × 3 days	Azithromycin	10 mg/kg/day in a single daily dose × 3 days

[a]Increasing reports of decreased susceptibility to quinolones. Concern that strains that have minimal inhibitory concentrations (MICs) >0.12–1 µg/mL to ciprofloxacin should be treated with an alternate drug, and this may require revising of the Clinical and Laboratory Standards Institute (CLSI) current breakpoint of ≤1 µg/mL.[102]

[b]Not approved for use in children.

Clinical illness, if left untreated, generally lasts 1 day to 1 month, with an average of 7 days. Although mortality is unusual in shigellosis, except in malnourished children and older adults, the clinical illness is more striking and more likely to lead to hospitalization than are most other forms of infectious diarrhea. Complications, which are unusual, generally consist of severe dehydration, febrile seizures, septicemia or pneumonia from coliform organisms (and, less commonly, the infecting *Shigella* strain), keratoconjunctivitis, immune complex acute glomerulonephritis, post-*Shigella* irritable bowel syndrome and reactive arthritis, and hemolytic uremic syndrome. *S. dysenteriae* 1 characteristically produces a more serious form of diarrhea, and the mortality associated with untreated disease during epidemics may be as high as 20%. Now that oral rehydration therapy has reduced the incidence of most cases of dehydration-associated deaths from diarrhea, shigellosis represents the most important form of fatal enteric illness in areas of high endemicity.[89]

CONTROL

Environmental Control

A safe water supply is important for the control of shigellosis and is probably the single most important factor in areas with substandard sanitation facilities.[90] Chlorination is another factor important in decreasing the incidence of all waterborne enteric bacterial infections. Insecticides are useful in decreasing the vector population during peak seasons, and a decrease in the incidence of shigellosis, but not salmonellosis, may be seen after their use.[42] At other times of the year, it may be helpful to attack breeding places of insects. Garbage collection and disposal of excreta and sewage may also be useful in controlling the vectors.

In many areas of the developing world, it is necessary to examine the techniques of home preparation and storage of food. Important features may be improved, such as personal and food hygienic facilities, and refrigeration may be necessary. A major prerequisite in transmission in most cases of bacillary dysentery is the degree of contact and the level of personal hygiene between patients with disease and susceptible persons. Other factors are frequent and effective hand washing, voluntary removal of persons with diarrhea from roles as food handlers, and appropriate refrigeration and proper cooking of potentially infected foods. Breastfeeding is an important means of decreasing the incidence of bacillary dysentery in developing countries and in communities with substandard hygienic practices. Also, mothers should be taught how to prepare foods to supplement breastfeeding and to ensure the safety of the diet after weaning, thus improving sanitation and nutrition. Finally, cases of diarrhea should be adequately diagnosed and patients isolated, and antimicrobial therapy should be instituted in cases of bacillary dysentery to decrease the reservoir of virulent strains.

Immunologic Control

Epidemiologic studies have indicated that a degree of homologous immunity can be demonstrated in those who have recovered from bacillary dysentery.[91–93] These observations have supported the idea that a protective vaccine might be developed. It was shown that killed parenteral vaccines fail to protect animals against experimentally produced shigellosis[94] and to protect humans against naturally occurring illness.[95] Besredka[96] suggested that the immunity against bacillary dysentery conferred by one attack of the disease was essentially the result of sensitization of the intestinal mucosa to dysentery bacilli and that the antibodies circulating in serum had a small role or none at all in protection. After more than 90 years, Besredka's concept of intestinal immunity is still held as the primary mode through which immunologic control might be feasible. However, the nature of the intestinal immune response has not been completely characterized. In natural shigellosis, immunoglobulin A (IgA) concentrations in stool increase, as do anti-*Shigella* secretory IgA antibodies directed to homologous lipopolysaccharide.[97] Also, lymphocytes, monocytes, and granulocytes, in the absence of complement but in the presence of antibody, may serve an anti-*Shigella* function through cell-mediated mechanisms.[98]

The most successful outcome in the area of *Shigella* vaccine development was achieved by Mel and colleagues,[99] who used streptomycin-dependent mutant strains of *Shigella* as orally administered immunizing agents in Yugoslavian army soldiers and in children living in areas of hyperendemicity. These investigators demonstrated that immunization with a live-attenuated bacterial strain given orally in multiple doses (at least four) would prevent clinical disease but not alter the carrier status, provided that gastric acidity was first decreased with sodium bicarbonate swallowed just before the vaccine was given. Serotype-specific protection followed vaccination and lasted for at least 6 months, and the immunizing agent remained protective when combined as a bivalent preparation. Experiments in volunteers have demonstrated that the protective immunity imparted by oral immunization approximates that after recovery from disease.[100]

In the future, immunologic control may be possible against a limited number of serotypes of shigellae when attack rates are shown to be particularly high. Further research is being directed toward developing an immunizing strain that multiplies in the intestinal tract so that fewer doses need to be administered.[101] Attenuated bacteria can be constructed that are better adapted to host intestinal proliferation and that combine multiple serotypes. Avirulent mutants and bioengineered strains may produce anti-*Shigella* immunity. Conjugate *Shigella* vaccines are also being evaluated. It is possible that antitoxin immunity might be important to susceptibility and that a successful immunizing agent should also include a toxoid component.

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225 *Haemophilus* Species, Including *H. influenzae* and *H. ducreyi* (Chancroid)

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SHORT VIEW SUMMARY

Definition

- *Haemophilus* is a gram-negative coccobacillus with fastidious growth requirements.
- Nontypeable (nonencapsulated) *Haemophilus influenzae* is a common cause of otitis media in children and exacerbations of chronic obstructive pulmonary disease (COPD) in adults. Invasive infections due to *H. influenzae* are now more commonly due to nontypeable than typeable isolates in countries in which children are vaccinated against *H. influenzae* type b. Invasive infections with nontypeable *H. influenzae,* usually bacteremic pneumonia, most often occur in neonates or persons younger than 2 or older than 55 years with underlying comorbidities.
- Encapsulated *H. influenzae* type b causes invasive infections, including meningitis and epiglottitis, in children younger than 6 years. The other five serotypes are rare causes of invasive infection.
- *Haemophilus ducreyi* causes chancroid, a genital ulcer disease that facilitates human immunodeficiency virus transmission. Ulcers are painful and may be accompanied by inguinal lymphadenopathy, which may be suppurative. Chronic cutaneous ulcers outside the genital area occur in tropical countries.
- *Haemophilus haemolyticus* and other *Haemophilus* species are unusual causes of human disease.

Epidemiology

- The ecologic niche of *H. influenzae* is the human respiratory tract.
- Colonization of the nasopharynx by nontypeable *H. influenzae* begins in infancy, is common during childhood, and persists at a lower rate in adults.
- Invasive *H. influenzae* type b infections are rare in regions of the world where *H. influenzae* type b conjugate vaccines are used widely but are prevalent in regions where the vaccine is not used.
- The prevalence of genital chancroid appears to be decreasing, but nongenital skin ulcers have been reported in tropical countries, based on polymerase chain reaction (PCR) detection.

Microbiology

- Many members of the genus *Haemophilus* are part of the normal bacterial flora of the human upper respiratory tract.
- Fastidious growth requirements are used to distinguish the species in the laboratory. *H. influenzae* strains have six serotypes and eight biotypes. *H. ducreyi* requires special medium for culture and is better detected in genital lesions with PCR assay.

Diagnosis

- Invasive infections caused by *H. influenzae* are established by isolating the organism from sterile fluid or, with *H. influenzae* type b, by detecting capsular antigen in cerebrospinal fluid or blood. Chancroid is usually diagnosed clinically, although painful genital ulcers can be misdiagnosed as herpes simplex.

Therapy

- Otitis media and exacerbations of COPD are treated with oral antimicrobial agents, with the choice generally guided by expert guidelines.
- *H. influenzae* type b meningitis is treated with ceftriaxone, 75 to 100 mg/kg divided into 12- hour doses, or cefotaxime, 200 mg/kg/day, divided into 6-hour doses. Dexamethasone is also administered, 0.6 mg/kg/day IV in four divided doses for 4 days to children older than 2 months.
- Chancroid is treated with azithromycin, 1 g orally (single dose), or ceftriaxone, 250 mg IM (single dose). Alternatives are ciprofloxacin 500 mg twice daily for 3 days or erythromycin base 500 mg three times a day for 7 days.

Prevention

- All children should receive the *H. influenzae* type b conjugate vaccine series beginning at age 2 months as part of routine childhood vaccination.
- Incompletely immunized household contacts of patients with meningitis should receive rifampin prophylaxis (20 mg/kg once daily [600 mg maximum] for 4 days).
- Sexual contacts of patients with chancroid within 10 days of symptom onset should be treated, even if asymptomatic.

HAEMOPHILUS INFLUENZAE

Description of the Pathogen

H. influenzae is a small, nonmotile, non–spore-forming bacterium and a pathogen of humans found principally in the upper respiratory tract, first reported by Pfeiffer in 1892. The sensational claim that it was the primary agent of epidemic influenza proved fallacious; nonetheless, it has a wide range of pathogenic potential. Its requirement for growth factors, which can be supplied by erythrocytes, accounts for the generic name *Haemophilus* ("blood-loving"). In microscopic appearance, it is a small (1×0.3 μ) gram-negative bacterium. Stained organisms obtained from clinical specimens vary microscopically from small coccobacilli to long filaments. This variable morphologic appearance (pleomorphism) and inconsistent uptake of dyes (e.g., safranin) may result in erroneous interpretations of stained smears.

Aerobic growth of *H. influenzae* requires two supplements known as X factor and V factor, although neither refers to a single substance. X factor can be supplied by heat-stable iron-containing pigments that supply protoporphyrins. Porphyrin-based assays represent the most reliable methods for identifying *Haemophilus* species.[1] Because X factor is not required for anaerobic growth of *H. influenzae,* confusion may arise if *H. influenzae* is grown anaerobically (e.g., after stab inoculation). The heat-labile V factor, a coenzyme, may be supplied by nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, or nicotinamide nucleoside. Although present in erythrocytes, V factor must be released from the cell to sustain optimal growth, and thus standard blood agar is an unsatisfactory medium. *H. influenzae* exhibits satellitism around colonies of hemolytic *Staphylococcus aureus* (a source of V factor), and this technique may be used to identify *H. influenzae.* Although it

is not a strict requirement, some *H. influenzae* strains grow best in 5% to 10% carbon dioxide. Determining serotype by slide agglutination is customary, although polymerase chain reaction (PCR) assay for capsular genes has revealed false-positive slide agglutination tests.[2]

Distinguishing *Haemophilus influenzae* from *Haemophilus haemolyticus*

Strains of *H. haemolyticus* are frequently misidentified as *H. influenzae* in clinical microbiology laboratories and in published studies. The confusion results from the observation that many strains of *H. haemolyticus* are not hemolytic, and this is the sole characteristic used routinely to distinguish *H. haemolyticus* from *H. influenzae* in commercial kits and in clinical microbiology laboratories. *H. influenzae* and *H. haemolyticus* both have growth requirements for X and V factors. Analysis of 500 strains originally identified as nontypeable *H. influenzae* revealed that 27% of nasopharyngeal isolates from children and 40% of sputum isolates from adults were in fact *H. haemolyticus*.[3] *H. haemolyticus* is a commensal but does cause disease rarely.[3] Strains of *H. haemolyticus* and *H. influenzae* can be distinguished from one another through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry or differences in various genetic markers, including 16S ribosomal RNA (rRNA), superoxide dismutase C, outer membrane protein (OMP) P6, protein D, fuculose kinase, and others.[3–11] Because *H. influenzae* and *H. haemolyticus* share the human upper airway as their ecologic niche and are competent for DNA exchange, there appears to be an evolutionary continuum between the two species.

Biotypes

Viability of *H. influenzae* is lost rapidly, so clinical specimens should be inoculated onto appropriate media without delay. A biotyping scheme devised by Kilian (based on indole production, urease, and ornithine decarboxylase activity) may be used to characterize individual isolates.[12] Biotype III includes *Haemophilus aegyptius,* the "Koch-Weeks bacillus." A clone of biotype IV strains is associated with neonatal and postpartum infections.

Serotypes

Colonies of *H. influenzae* are usually granular, transparent (or slightly opaque), circular, and dome shaped. On chocolate agar, most colonies attain a size of about 0.5 to 0.8 mm during the first 24 hours of growth at 37°C, enlarging to 1.0 to 1.5 mm by 48 hours. Six serotypes, designated a to f, are based on antigenically distinct capsular polysaccharide types. Colonies of encapsulated strains are mucoid (iridescent when grown on transparent media and examined with an indirect source of light) and may attain a size of 3 to 4 mm. Capsular type b strains are important invasive pathogens in humans. Strains of *H. influenzae* that lack a polysaccharide capsule are generally referred to as nontypeable because they are nonreactive with typing antisera raised against each of the six capsules. The population structure of *H. influenzae* type b is clonal, whereas nontypeable strains demonstrate substantial genetic diversity.[13] Most unencapsulated isolates are not capsule-deficient variants of extant capsule clones; they are genetically distinct from encapsulated strains of *H. influenzae.*

Epidemiology and Respiratory Tract Colonization

H. influenzae is recovered exclusively from humans; no other natural host is known. It is recovered from the upper airway and, rarely, the genital tract. Spread from one person to another occurs by means of airborne droplets or of direct contact with secretions.

Colonization in Children

Exposure to nontypeable *H. influenzae* begins after birth. Colonization of the respiratory tract is a dynamic process, with new strains of nontypeable *H. influenzae* being acquired and cleared from the respiratory tract frequently.[14] Varied patterns of colonization are evident in the first 2 years of life: brief colonization with one strain, prolonged colonization with one strain, and recurrent colonization with different strains.[14–17] Children who attend daycare centers are colonized at a higher rate than control children.[15,18] Nasopharyngeal colonization by *H. influenzae* in the first year of life is associated with an increased risk of recurrent otitis media compared with children who remain free of colonization.[19,20] The widespread administration of pneumococcal polysaccharide vaccines has caused changes in patterns of nasopharyngeal colonization. A reduction in nasopharyngeal colonization by vaccine serotypes of *Streptococcus pneumoniae,* with "replacement" of vaccine serotypes of *S. pneumoniae* by nonvaccine pneumococcal serotypes, nontypeable *H. influenzae,* and *Moraxella catarrhalis* may be occurring.[21,22] A pneumococcal conjugate vaccine that contains protein D of *H. influenzae* is being administered widely in many countries globally. The vaccine induces partial protection against otitis media caused by nontypeable *H. influenzae* but appears to have no significant effect on reducing colonization by nontypeable *H. influenzae.*[23,24] Monitoring of colonization patterns as new vaccines for bacteria that reside in the nasopharynx are developed and administered will be important.

Colonization in Adults With Chronic Obstructive Pulmonary Disease

Nontypeable *H. influenzae* frequently colonizes the lower respiratory tract in the setting of chronic obstructive pulmonary disease (COPD) and cystic fibrosis; multiple strains colonize the respiratory tract of these patients simultaneously.[25–28] Acquisition of new strains of nontypeable *H. influenzae* is associated with an increased risk of exacerbations of COPD.[4,29,30] Use of selective media improves the recovery rate of *H. influenzae* from the sputum of patients with cystic fibrosis.[31]

Based on its binding to mucin and adherence to epithelial cells, *H. influenzae* has long been considered an extracellular pathogen. Several lines of evidence, however, have established that *H. influenzae* has both an extracellular and an intracellular niche in the human respiratory tract.[32–37] Therefore, *H. influenzae* is present in the airway lumen, bound to mucin, adherent to respiratory cells, found within the interstitium of the submucosa, and found within cells of the respiratory tract. This observation has important implications for understanding the dynamics of colonization of the human respiratory tract and the human immune response to the bacterium.

Colonization and Conjugate Vaccines

Before the widespread use of conjugate vaccines, type b strains colonized the nasopharynx of children at a rate of 2% to 4%. The rate of nasopharyngeal colonization by type b strains has decreased substantially with the use of conjugate vaccines to prevent invasive infections caused by *H. influenzae* type b. Table 225.1 summarizes several features of nontypeable and type b strains.

Pathogenesis

Otitis Media

The first step in the pathogenesis of infection is colonization of the upper respiratory tract. *H. influenzae* expresses a variety of adhesin

TABLE 225.1 Comparison of Selected Features of Nontypeable and Type b Strains of *Haemophilus influenzae*

FEATURE	NONTYPEABLE STRAINS	TYPE B STRAINS
Colonization rate in upper respiratory tract	30%–80%	<1% in vaccinated populations; 2%–4% in unvaccinated populations
Capsule	Unencapsulated	PRP capsule
Pathogenesis	Mucosal infections	Invasive infections
Clinical manifestations	Otitis media, exacerbations of COPD, sinusitis	Meningitis, epiglottitis, and other invasive infections in infants and children
Evolutionary history	Genetically diverse	Clonal
Vaccine	None available; under development	Highly effective PRP conjugate vaccines

COPD, Chronic obstructive pulmonary disease; *PRP,* polyribitol ribose phosphate.

TABLE 225.2 Adhesins of *Haemophilus influenzae*

ADHESIN	MOLECULAR MASS (KDA)	OBSERVATION
Pili (fimbriae)	20–25	*hifA-hifE* gene cluster
Type 4 pilus	≈14	*pilABCD* gene cluster; mediates twitching motility
HMW1 and HMW2	120–125	Homologous with filamentous hemagglutinin of *Bordetella pertussis*
Hap	155	Homologous with IgA protease
Hsf	≈240	Surface fibrils; present in type b strains; homologue of Hia
Hia	115	Hia absent from strains that express HMW1, HMW2; present in nontypeable strains
OMP P5	≈35	Binds mucin; also called fimbrin; homologous with OMP A of *Escherichia coli*
OMP P2	36–42	Binds mucin and laminin
PE-binding adhesin	46	Binds phosphatidylethanolamine
Protein F	≈30	Binds laminin and respiratory epithelial cells
Protein E	16	Binds myeloma IgD, laminin, vitronectin and type 2 alveolar cells
Lipooligosaccharide	2.5–3.3	Adhesin for respiratory epithelial cells

Hap, Haemophilus adhesin and penetration; *Hia, H. influenzae* adhesin; *HMW,* high molecular weight; *Hsf, Haemophilus* surface fibrils; *IgA,* immunoglobulin A; *IgD,* immunoglobulin D; *OMP,* outer membrane protein; *PE,* phosphatidylethanolamine.

molecules (Table 225.2), each of which has its own specificity for host receptors.[38–44] The prevalence and distribution of adhesins vary among nontypeable strains.[42,45] In contrast to type b strains, which gain access to the bloodstream, nontypeable strains cause disease by local invasion of mucosal surfaces. The pathogenesis of otitis media involves direct extension of bacteria from the nasopharynx to the middle ear via the eustachian tube.[46] Release of lipooligosaccharide, lipoproteins, peptidoglycan fragments, and other antigens induces host inflammation.

Strains of nontypeable *H. influenzae* show differences in their pathogenic potential. A subset of strains that colonize the nasopharynx is capable of causing otitis media and has different sets of genes compared with strains that cause asymptomatic colonization. For example, otitis media strains are more likely to have the lipooligosaccharide synthesis gene *lic2B,* the histidine operon, and the urease operon than are asymptomatic colonizing strains.[47–49] Nontypeable *H. influenzae* rapidly and reversibly regulates the expression of many virulence genes through phase variation of methyltransferase proteins, which methylate DNA at sequence-specific sites, leading to changes in expression of many genes. This phasevarion switching plays an important role in the pathogenesis of nontypeable *H. influenzae* otitis media in the chinchilla model.[50]

Otitis Media With Effusion

Nontypeable *H. influenzae* has also been implicated as a cause of otitis media with effusion, a term that refers to the presence of middle ear fluid in the absence of clinical signs of acute otitis media. In addition to positive cultures of some middle ear fluids, analysis with PCR reveals the presence of microbial DNA and messenger RNA (mRNA), suggesting that *H. influenzae* is present in a viable but nonculturable form in some cases of otitis media with effusion.[51,52]

Biofilms

H. influenzae in the form of biofilms is present in the middle ear in animal models and in the middle ears of children with otitis media.[53–58] A biofilm is a community of bacteria encased in a matrix and attached to a surface. Bacteria in biofilms are more resistant to host clearance mechanisms and more resistant to antibiotics compared with planktonic bacteria.[59] Nontypeable *H. influenzae* biofilms are associated with recurrent and chronic otitis media.[53]

Exacerbations of Chronic Obstructive Pulmonary Disease

The lower respiratory tract of adults with COPD is chronically colonized by nontypeable *H. influenzae.* The course of COPD is characterized by intermittent exacerbations of the disease. Several lines of evidence implicate *H. influenzae* as the most common bacterial cause of exacerbations, including bronchoscopic sampling of the lower respiratory tract during exacerbations, analysis of immune responses to *H. influenzae* isolated from patients experiencing exacerbations, correlation of airway inflammation with sputum bacteriology, and molecular analysis of prospectively collected isolates.[29,30,60] Differences in pathogenic potential among strains in the setting of COPD are based on genome content.[61,62] A complex host-pathogen interaction most likely determines the outcome of the acquisition of a new strain of nontypeable *H. influenzae*; the determinants include virulence of the strain, degree of host impairment of innate immunity and pulmonary function, host inflammatory response, preexisting immunity, perception of symptoms, and other factors.[30] Strains of nontypeable *H. influenzae* that persist in COPD airways regulate expression of critical virulence functions with slipped-strand mispairing, mediated by changes in simple sequence repeats in multiple genes as a major mechanism for survival in the hostile environment of the human airways.[63]

Invasive Infections Caused by *Haemophilus influenzae* Type b

The importance of the type b capsule as a critical virulence factor in the pathogenesis of invasive disease has been well established with the use of genetic techniques and an infant rat model of bacteremia and meningitis.[64] Mutants lacking the polyribitol ribose phosphate (PRP) capsule do not cause invasive disease, whereas the isogenic parent strains are highly virulent in the infant rat model. The type b capsular polysaccharide is composed of PRP. The capsule enables the organism to invade the bloodstream after colonization of the respiratory tract (see later).

Immunity

Nontypeable *Haemophilus influenzae*

Immunity to infection by nontypeable *H. influenzae* is complex and not completely understood. A hallmark of noninvasive infections caused by nontypeable *H. influenzae* is their propensity for recurrence. The immune response to surface antigens of nontypeable strains is intimately involved in the pathogenesis of recurrent infection. Studies in animal models, in adults with COPD, and in children with otitis media have all demonstrated that the most prominent antibody response is directed at strain-specific determinants.[65–67] The clinical observation of recurrent infections in immunocompetent hosts (recurrent otitis media in children and recurrent exacerbations in COPD) suggests that strain-specific immune responses leave the host susceptible to recurrent infections by different strains of *H. influenzae.* A variety of membrane-associated surface-exposed determinants are immunogenic and potential targets of protective host immune responses. For example, OMP P2, the major porin protein, contains immunodominant strain-specific determinants on the bacterial surface. Adults with COPD make potentially protective antibodies to strain-specific determinants on P2 after infection. Patients remain susceptible to recurrent infections by other strains. Furthermore, the P2 genes of strains that colonize adults with COPD undergo point mutations in the human respiratory tract.[68,69] The mutations result in amino-acid changes in the surface-exposed loops of the P2 molecule. A similar phenomenon has been observed with OMP P5.[70] These variants have a selective advantage and are able to evade the host response and cause recurrent or persistent infection.

The presence of serum bactericidal antibody is associated with protection from otitis media caused by nontypeable *H. influenzae,* as is serum antibody to protein D.[66,71] Because nontypeable *H. influenzae* causes mucosal infection, mucosal immunity likely plays a role in host defense; however, the mucosal immune response to *H. influenzae* is

poorly understood. Finally, observations have suggested that cell-mediated immune responses, including Th17 responses, play a role in protection against infection.[72–74]

Haemophilus influenzae Type b

Protection against invasive *H. influenzae* type b infections is mediated by antibodies to the type b capsular polysaccharide PRP. Serum anti-PRP antibodies activate complement-mediated bactericidal and opsonic activity in vitro and mediate protective immunity against systemic infections in humans. The level of maternally acquired serum antibody to PRP declines after birth and reaches a nadir at approximately 18 to 24 months of age, the peak age incidence of meningitis caused by *H. influenzae* type b in an unimmunized child. The level of antibody to PRP then gradually rises, apparently as a result of exposure to *H. influenzae* type b or cross-reacting antigens. Systemic disease is unusual after the age of 6 years even in the absence of immunization, because of, at least in part, naturally acquired antibody to PRP.

Immunization with vaccines that are composed of PRP conjugated to carrier proteins affords protection by inducing antibodies to PRP. These vaccines are now widely used and are highly effective in preventing invasive disease caused by *H. influenzae* type b in infants and children.[75–77] In addition, these vaccines prevent colonization of the nasopharynx; this effect accounts for herd immunity by reducing the circulation of type b strains.

Clinical Manifestations of Nontypeable *Haemophilus influenzae*

Otitis Media

Nontypeable *H. influenzae* accounts for 25% to 35% of all cases of acute otitis media. Approximately 16 million episodes of otitis media occur annually in the United States. Although such episodes occur at any age, they are most common in children aged 6 months to 5 years. The typical clinical presentation of acute otitis media in infants is fever and irritability; older children also complain of ear pain. A prior viral respiratory tract infection is commonly the antecedent of an episode of otitis media. The diagnosis is made with pneumatic otoscopy. A precise causative diagnosis requires tympanocentesis, but this is not performed routinely.

Although it is not possible to determine the causative agent of otitis media in an individual child based on clinical characteristics, certain features are associated with otitis media caused by nontypeable *H. influenzae* compared with *S. pneumoniae.* Otitis media caused by nontypeable *H. influenzae* is less likely to cause fever and is less often associated with otorrhea than with pneumococcal otitis media, suggesting that the former causes a less virulent form of the disease, but substantial overlap is seen.[78,79] Nevertheless, features associated with nontypeable *H. influenzae* otitis media include a history of recurrent episodes, treatment failure, concomitant conjunctivitis, previous amoxicillin treatment, bilateral otitis media, and acute otitis media within 2 weeks of completing a course of any antibiotic.[80–83] Since 2000, infants in many countries have received the 7-valent pneumococcal conjugate vaccine, and since 2010, the 10-valent and 13-valent vaccines. These vaccines appear to have resulted in an overall decrease in otitis media and complications from otitis media in widely immunized populations.[84–86] However, an increase in the proportion of acute otitis media caused by nontypeable *H. influenzae* in children in whom initial antimicrobial therapy fails or who have recurrent episodes has occurred coincident with the widespread use of these vaccines.[87,88] Continued monitoring of the incidence and etiology of otitis media will be critical.

Exacerbations of Chronic Obstructive Pulmonary Disease

The course of COPD is characterized by intermittent exacerbations of the disease. It is estimated that approximately half of exacerbations are caused by bacteria, and nontypeable *H. influenzae* is the most common bacterial cause.[4,29] The three cardinal signs of an exacerbation are an increase from baseline of sputum production, sputum purulence (change in sputum color), and dyspnea. Fever is generally absent or low grade and infiltrates are not present on chest radiographs. A sputum Gram strain often reveals abundant gram-negative coccobacilli.

Community-Acquired Pneumonia

Nontypeable *H. influenzae* is an important cause of pneumonia in adults, particularly in older adults and those with COPD and acquired immunodeficiency syndrome.[89,90] The clinical features are indistinguishable from those of pneumonia caused by other bacteria and include fever, cough, and purulent sputum, usually of several days' duration. The chest film reveals infiltrates that may be patchy or show lobar distribution. A Gram-stained smear of the sputum shows a predominance of small gram-negative coccobacilli.

Acute Respiratory Tract Infections in Children in Developing Countries

In countries in which adverse socioeconomic circumstances are prevalent, acute pneumonia in infants is a major cause of morbidity and mortality. Nontypeable *H. influenzae* likely accounts for a significant proportion of these cases of pneumonia, but further studies are needed to establish the etiologic agents. The importance of acute respiratory tract infections as a major global health problem has led to the establishment of international programs, with the aim of enhancing the recognition, appropriate management, and prevention of respiratory tract infections.

Sinusitis

Studies that have used cultures of direct sinus aspirates have shown that nontypeable *H. influenzae* is a common cause of acute maxillary sinusitis.[91,92] Patients experience nasal obstruction, purulent nasal discharge, headache, and facial pain. As in the case of otitis media, an invasive procedure (sinus aspiration) is required to establish a causative diagnosis.

Neonatal and Maternal Sepsis

Neonatal sepsis caused by nontypeable *H. influenzae* is associated with 50% mortality overall and 90% mortality in premature infants. Many strains that cause neonatal sepsis are biotype IV and share several genotypic and phenotypic characteristics with one another.[93] Indeed, studies of the genetic relationships of these potentially invasive strains and other nontypeable strains have suggested that the invasive biotype IV strains are closely related to *H. haemolyticus.*[4]

These same biotype IV strains also cause postpartum sepsis associated with endometritis. Nontypeable *H. influenzae* is a well-documented cause of tubo-ovarian abscess or chronic salpingitis. Diagnosis is established through tubal cultures at laparoscopy or cultures of peritoneal fluid obtained with culdocentesis.

Bacteremia and Invasive Infections

Although the most common clinical manifestations of infections by nontypeable *H. influenzae* are otitis media and nonbacteremic respiratory tract infections in adults, the organism also occasionally causes bacteremia. Most invasive *H. influenzae* infections in countries in which the *H. influenzae* type b vaccines are used are caused by nontypeable strains.[94–100] Although these vaccines have changed the proportion of nontypeable strains that cause invasive disease by preventing disease from type b strains, there is no convincing evidence that an increased incidence of invasive disease from nontypeable strains has occurred.[101] However, close monitoring will be important. Population-based studies have estimated an incidence of 1.7 cases per 100,000 adults of invasive disease caused by *H. influenzae,* and overall the incidence is highest in older adults.[98,102] Most people with bacteremia have underlying conditions, such as alcoholism, cardiopulmonary disease, human immunodeficiency virus (HIV) infection, or cancer. The respiratory tract is the usual source of infection when bacteremia is present. Invasive strains of nontypeable *H. influenzae* are genetically and phenotypically diverse.[103] Bacteremic infections caused by nontypeable *H. influenzae* are associated with significant mortality.

Nontypeable *H. influenzae* is also an unusual cause of a variety of invasive infections that have been documented in case reports and small series. All the invasive diseases that are commonly caused by *H. influenzae* type b are, on occasion, caused by nontypeable strains and by types a, c, d, e, and f. These infections include adult epiglottitis, empyema, septic arthritis, cellulitis, osteomyelitis, pericarditis, cholecystitis, intraabdominal infection, and vascular graft infection.

Conjunctivitis

Nontypeable *H. influenzae* is the most common bacterial cause of conjunctivitis in children.[104,105] In contrast to the sporadic nature of other *Haemophilus* infections, conjunctivitis can occur in outbreaks, particularly in daycare centers. Clinical features include conjunctival hyperemia and purulent discharge. On occasion, nontypeable *H. influenzae* causes severe conjunctivitis that is characterized by copious, purulent discharge, lid edema, chemosis, and keratitis.

Clinical Manifestations of *Haemophilus influenzae* Type b

Meningitis

Meningitis is the most serious acute manifestation of systemic infection caused by *H. influenzae.* Antecedent symptoms of upper respiratory infection are common. Specific questioning concerning the occurrence of disease in contacts (household, daycare centers) is prudent. None of the clinical features of meningitis caused by *H. influenzae* distinguishes it from other forms of purulent meningitis. The peak age incidence varies somewhat among populations, depending in part on vaccine use, but this infection now occurs most often in those who are incompletely immunized. Cases in adults are infrequent and often involve a background of recent or remote head trauma, prior neurosurgery, paranasal sinusitis, otitis, or cerebrospinal fluid (CSF) leak. *H. influenzae* meningitis in neonates is also rare, but such cases can resemble early-onset group B streptococcal infection. The most common signs are fever and altered central nervous system function, but the young child may have few specific signs, and nuchal rigidity is often absent. More obvious manifestations, such as seizures or coma, commonly develop as the disease progresses. Subdural effusions are a common complication. Clinical suspicion should be greatest when after 2 or 3 days of adequate therapy there is a tense anterior fontanelle, seizures (particularly if focal), hemiparesis, or neurologic deterioration. In older children, the clinician looks for papilledema and altered mental status.

With appropriate management, the overall mortality rate from *H. influenzae* meningitis is less than 5%, but apparently permanent sequelae occur in many of the survivors.

Epiglottitis

Acute respiratory obstruction caused by cellulitis of the supraglottic tissues is a potentially lethal disease with a characteristically fulminating onset. Swelling of the epiglottis and aryepiglottic folds with complete obliteration of the vallecular and piriform sinuses is typical. Usually, the patient is a child (aged 2–7 years), but occurrence in adults is also well known. The onset is often explosive, with initial features being sore throat, fever, and dyspnea, progressing rapidly to dysphagia, pooling of oral secretions, and drooling of saliva from the mouth. The child is restless and anxious and adopts a sitting position, with neck extended and chin protruding to reduce airway obstruction. Abrupt deterioration commonly occurs within a few hours, resulting in death in the absence of adequate treatment. The characteristic findings are seen above the larynx. The epiglottis is red and swollen and bears a striking resemblance to a bright red cherry obstructing the pharynx at the base of the tongue. The trachea appears normal. Examination of the larynx should be performed only in a setting in which an airway can be placed, because this examination, if injudiciously performed, may lead to fatal respiratory obstruction. A lateral radiograph of the oropharynx can be useful in visualizing the swollen epiglottis.

Pneumonia and Empyema

The true frequency of primary lung infections caused by *H. influenzae* type b in children is difficult to determine with accuracy. Typically, the patient is between 4 months and 4 years of age and becomes ill in winter or spring, presenting with a consolidative pneumonia (often with pleural involvement) that is severe enough to necessitate hospitalization. In a series from England and Wales, 52% of 214 bacteremic children with nontypeable *H. influenzae* were younger than 2 years, and 52% had a comorbidity.[106] The only clinical feature that tends to distinguish *H. influenzae* pneumonia from bacterial pneumonias caused by *S. aureus* or *S. pneumoniae* is a more insidious onset. The development of severe dyspnea, tachycardia, and evidence of cardiovascular failure suggests pericarditis, an uncommon but important complication.

Cellulitis

Cellulitis is predominantly seen in young children. The clinical features are fever and a raised, warm, tender area of distinctive reddish-blue hue, most often located on one cheek or in the periorbital region. The distinctive color, its location, and the age of the child should suggest the cause. The soft tissue involvement progresses rapidly over a few hours. Some of these children have, or develop, evidence of other septic foci (e.g., meningitis) because an accompanying bacteremia is extremely common.

Bacteremia Without Localized Disease

Children, particularly those 6 to 36 months of age, may acquire bacteremia without evidence of local disease; *S. pneumoniae* is the most common cause of this syndrome, although *H. influenzae* can also be seen. Typically, fever, anorexia, and lethargy prompt the visit to a physician; the examination is nondiagnostic. This condition is appreciated most often in those with a temperature higher than 102°F (39°C) and an increased peripheral neutrophil count. Children with sickle cell disease or with a previous splenectomy are particularly susceptible. Early diagnosis and therapy are critical because these patients may worsen rapidly and develop septic shock or a localized purulent focus.

Septic Arthritis

H. influenzae causes septic arthritis in children younger than 2 years. Typically, there is involvement of a single, large, weight-bearing joint (without osteomyelitis), displaying decreased mobility, pain on movement, and swelling. Positive cultures of blood and joint fluid are usual. However, the signs and symptoms may be more subtle; for example, septic arthritis is an important cause of prolonged fever and irritability (or prolonged antigenemia) during the treatment of other systemic *H. influenzae* diseases (e.g., meningitis).

Response to systemic antibiotics is dramatic and often curative, but long-term follow-up is important because residual joint dysfunction occurs in a significant percentage of children.

H. influenzae septic arthritis also occurs in adults. A review of 29 adults with *H. influenzae* arthritis found that 14 had multiarticular disease and 15 monoarticular disease, with 6 cases being in the knee only.[107] Nineteen patients also had extraarticular infection, including meningitis, pneumonia, sinusitis, and cellulitis. Predisposing factors were found in 22, such as alcohol abuse, trauma, rheumatoid arthritis, systemic lupus erythematosus, diabetes mellitus, splenectomy, multiple myeloma, lymphoma, or common variable hypogammaglobulinemia.

Clinical Manifestations of Non–Type b Encapsulated *Haemophilus influenzae*

Although non–type b encapsulated strains of *H. influenzae* (types a, c, d, e, and f) are unusual causes of invasive infection manifesting predominantly with bacteremia and pneumonia, the incidence of types a, e, and f infections has shown modest recent increases. Invasive *H. influenzae* type a infections are seen with increased frequency in indigenous populations of North America. The type a strains that cause invasive disease are clonal. Most infections due to non–type b encapsulated strains occur in the setting of underlying conditions.[108–110]

Diagnosis

Nontypeable *Haemophilus influenzae*

Because nontypeable *H. influenzae* is often present in the human upper airway in the absence of clinical disease, determining the causative pathogen in individual patients is challenging. A diagnosis of otitis media is made with pneumatic otoscopy. Culture of middle ear fluid obtained with tympanocentesis would be required in order to determine the microbial cause. However, because tympanocentesis is a relatively invasive procedure, empirical therapy with antibiotics is initiated based on predictions of the likely pathogens, which are known to be *S. pneumoniae,* nontypeable *H. influenzae,* and *M. catarrhalis* as determined in studies that have used cultures of middle ear fluid obtained with tympanocentesis. Judicious use of tympanocentesis in children with

recurrent or refractory otitis media may be indicated in order to identify the pathogen precisely in difficult cases, but this procedure is not used routinely.[111]

The cause of exacerbations of COPD and community-acquired pneumonia in individual patients is difficult to determine. The presence of nontypeable *H. influenzae* in the sputum of patients experiencing an exacerbation of COPD or pneumonia is suggestive of the diagnosis but does not establish the organism as the pathogen because it may be present in the airways in the absence of disease. Nontypeable *H. influenzae* is the most common bacterial cause of exacerbations of COPD and causes a smaller proportion of cases of community-acquired pneumonia.

Isolating the organism from a blood culture unequivocally establishes the organism as causative. Although blood cultures are invaluable when positive, most infections caused by nontypeable *H. influenzae* are not associated with bacteremia, so blood cultures are relatively insensitive.

Haemophilus influenzae Type b

A provisional diagnosis of meningitis, epiglottitis, facial cellulitis, or septic arthritis is usually prompted by the history and clinical findings. Confirmation requires microbiologic studies. Cultures of blood, CSF, and other normally sterile fluids (e.g., from joints or pleural, subdural, or pericardial spaces) are diagnostic. Even if antibiotic therapy has been started, the yield is sufficiently great to recommend that they be taken. Cultures of the inflamed epiglottis are generally positive, but specimens should be taken only when a functional airway can be guaranteed. Whenever feasible, specimens obtained for culture should also be Gram stained; in about 70% of cases of meningitis, CSF smears reveal typical organisms. Detection of capsular antigen in serum, CSF, or concentrated urine with immunoelectrophoresis, latex agglutination, or enzyme-linked immunosorbent assay may be diagnostic, and diagnosis can be made in up to 90% of culture-proven cases of meningitis. Antigen is also often detected in infected pleural, pericardial, or joint fluid and can facilitate diagnosis because it persists after antibiotic therapy.

Therapy

Nontypeable *Haemophilus influenzae*

Many infections caused by nontypeable *H. influenzae,* such as otitis media and exacerbations of COPD, can be treated with oral antimicrobial agents. Overall, approximately 30% of nontypeable strains produce β-lactamase, but substantial geographic variability in this rate is observed. Therefore, ampicillin and amoxicillin should be used only if the susceptibility of the infecting isolate is known. Mutations in the *fts1* gene, which codes for penicillin-binding protein 3, is a more recently recognized mechanism of resistance in *H. influenzae* and *H. haemolyticus* isolates.[10,112] This mechanism is common in Japan and increasing in prevalence in Europe. Although it is still uncommon in the United States, careful vigilance will be important. The clinician who manages patients with otitis media and exacerbations of COPD frequently chooses an antimicrobial agent empirically. In this circumstance, the antimicrobial agent should be active against *S. pneumoniae* and *M. catarrhalis* in addition to *H. influenzae.*

Oral antimicrobial agents that are active against nontypeable *H. influenzae* include amoxicillin-clavulanate, fluoroquinolones, macrolides (e.g., azithromycin, clarithromycin), and various extended-spectrum cephalosporins (e.g., cefixime, cefpodoxime, cefprozil, cefaclor, cefuroxime, and others).

Parenteral antibiotic therapy is indicated for more serious infections caused by nontypeable *H. influenzae.* Parenteral antimicrobial agents that are active include cephalosporins (e.g., ceftriaxone, cefuroxime, ceftazidime, cefotaxime), ampicillin-sulbactam, and fluoroquinolones.

Those with certain immunodeficiencies, especially those with primary deficiency of antibody synthesis, have increased susceptibility to infection, especially with nontypeable *H. influenzae.* They may benefit from passive infusion of immunoglobulin preparations administered intramuscularly or intravenously. This form of immunoglobulin replacement decreases the incidence of systemic infections in these persons and the number of episodes of both upper and lower respiratory tract infections caused by nontypeable *H. influenzae.*

Haemophilus influenzae Type b

Without treatment, infection caused by *H. influenzae* type b can be rapidly fatal. This is particularly true of meningitis and epiglottitis. The most favored regimen is cefotaxime or ceftriaxone. For children, cefotaxime is given as 200 mg/kg/day, divided into 6-hour doses. The pediatric dose of ceftriaxone is 75 to 100 mg/kg, divided into 12-hour doses. Adult doses are ceftriaxone, 2 g every 12 hours, or cefotaxime, 2 g every 4 to 6 hours. Treatment is continued until the patient is afebrile and without clinical or laboratory signs of infection for 3 to 5 days. The usual duration of therapy is 7 to 10 days. Patients with complications, such as endophthalmitis, endocarditis, pericarditis, or osteomyelitis, may require 3 to 6 weeks of therapy.

Administration of corticosteroids to patients with *H. influenzae* type b meningitis reduces the incidence of deafness and perhaps other neurologic sequelae (see Chapter 87). The presumed mechanism is the reduction of inflammation that results from release of bacterial cell wall fragments when bacteria are killed by antibiotics. Dexamethasone therapy (0.6 mg/kg/day IV in four divided doses for 4 days) should be administered to children older than 2 months.

Antibiotic therapy is only one facet of the management of the child with *H. influenzae* infection. Critical attention must also be given to supportive therapy, including maintaining oxygenation and adequate perfusion of tissues.

Chemoprophylaxis for *Haemophilus influenzae* Type b

In the absence of prior immunization, household contacts of those younger than 4 years with invasive *H. influenzae* type b infection have a substantial incidence of disease. Rifampin prophylaxis as 20 mg/kg once daily (600 mg maximum) for 4 days has eradicated the carrier state in approximately 95% of carriers and significantly reduced the incidence of secondary cases in household members. Rifampin comes in 150- and 300-mg capsules. The dose can be conveniently given to young children in applesauce.

Rifampin prophylaxis is recommended for all household members, including adults (except pregnant women), when there has been contact with an index case of *H. influenzae* type b disease by a household member who is younger than 48 months and whose immunization status with the conjugate vaccine is incomplete, or who is an immunocompromised child of any age. A contact is defined as a child who is a household member or who has spent 4 or more hours each day with the index patient for at least 5 of the 7 days preceding the day on which the index patient was hospitalized. Based on efficacy of the *H. influenzae* type b vaccine, chemoprophylaxis is not recommended when all household contacts younger than 48 months have completed their immunization series. Children who were immunosuppressed at the time of vaccination may not have responded and therefore should be considered unvaccinated. If rifampin is to be effective in preventing secondary cases, it should be given within 7 days after the index patient is hospitalized. The index patient should also be given rifampin if treated with regimens other than cefotaxime or ceftriaxone. Chemoprophylaxis is usually provided just before discharge from the hospital.

Rifampin prophylaxis is indicated for all attendees and personnel at a daycare center or nursery when two or more cases of invasive *H. influenzae* type b disease have occurred within 60 days if incompletely immunized children attend the facility. The duration and dose of rifampin are the same as for household contacts. Chemoprophylaxis is not indicated for a single case at a daycare center or nursery.

Active Immunization Against *Haemophilus influenzae* Type b

Conjugate vaccines for invasive *H. influenzae* type b infections in infants and children are highly effective. The vaccines induce serum antibody to the PRP capsule; this antibody is bactericidal for the organism. The protective level of serum antibody to PRP has been estimated to be approximately 0.15 μg/mL, although this estimate must be interpreted cautiously. Vaccination reduces or eliminates carriage of *H. influenzae* type b strains, and this effect has played an important role in the effectiveness of the vaccine. The widespread use of conjugate vaccines has almost eradicated invasive disease in children younger than 5 years in countries

TABLE 225.3 *Haemophilus influenzae* Type b Conjugate Vaccines

SCIENTIFIC NAME	BRAND NAME (MANUFACTURER)	CARBOHYDRATE	PROTEIN CARRIER	POLYSACCHARIDE-TO-PROTEIN RATIO	RECOMMENDED DOSE CARBOHYDRATE (μG)
PRP-T[a]	ActHIB (Sanofi Pasteur) HIBERIX (GlaxoSmithKline)	Native PRP	Tetanus toxoid	0.33	10
PRP-OMPC[a]	PedvaxHIB (Merck)	Native PRP	OMPC	0.05–0.10	15

[a]These two vaccines are also available as combination vaccines with other childhood vaccines. See Recommended Immunization Schedule for Children and Adolescents Aged 18 Years or Younger, United States, 2017 (www.cdc.gov/vaccines/acip).
HIB, H. influenzae type b; *OMPC,* outer membrane protein complex; *PRP,* polyribitol ribose phosphate.

with universal vaccination programs. These vaccines represent a dramatic success in disease prevention and health care cost savings.

Certain populations, including Native American and Native Alaskan children, show a persistently elevated rate of infection, even with widespread vaccination. Furthermore, localized populations with low vaccination rates contribute to the continued circulation of *H. influenzae* type b strains, despite a national vaccination rate higher than 90%. Therefore, continued surveillance, particularly in these high-risk populations, will be important. Determination of the serotypes of disease isolates will distinguish disease that results from lack of vaccination or vaccine failure from invasive disease caused by non–type b strains. A striking reduction in the incidence of invasive infections caused by *H. influenzae* type b has been seen in countries in which conjugate vaccines have been used widely. Through a global public-private partnership effort, the Vaccine Alliance, established in 2000, *H. influenzae* b (Hib) vaccine has reached an increasing number of resource-poor countries, saving millions of lives globally.

Two conjugate vaccines are currently licensed and available in the United States (Table 225.3). All children should be immunized with a conjugate vaccine beginning at 2 months of age. A primary series consisting of three doses at 2, 4, and 6 months of age (PRP-T) or two doses given at 2 and 4 months (PRP-OMPC), depending on the vaccine product, is recommended. After administration of the primary series, antibody titers decline, so an additional booster dose should be given between 12 and 15 months of age. Vaccines may be administered during visits when other vaccines are given. Adverse reactions are few; the most common are pain, redness, and swelling at the injection site.

There are no currently licensed vaccines for nontypeable *H. influenzae.* However, a randomized, prospective, placebo-controlled trial with a pneumococcal conjugate vaccine that contained protein D, a conserved surface protein of *H. influenzae* as a carrier, showed partial efficacy in prevention of nontypeable *H. influenzae* otitis media.[113] This vaccine is now used widely throughout the world. Based on active research in the area, additional progress in developing vaccines to prevent infections caused by nontypeable *H. influenzae* is anticipated.[114–118]

Haemophilus influenzae Biogroup *aegyptius*

H. influenzae biogroup *aegyptius* (the Koch-Weeks bacillus) has long been known to cause conjunctivitis. In 1984, a fulminant systemic illness characterized by purpura, peripheral necrosis, vascular collapse, and *H. influenzae* biogroup *aegyptius* bacteremia was described in a small Brazilian town.[119] The mortality was 70%, but subsequent reports described milder forms of the illness. The illness, called Brazilian purpuric fever, was described in several rural Brazilian towns in addition to two cases in Australia. The clinical illness has not been reported since the early 1990s.

H. influenzae biogroup *aegyptius* is remarkable in that the organism acquired the capacity to cause a fulminant invasive disease despite lacking a capsule, which is often associated with invasive infections. Strains of *H. influenzae* biogroup *aegyptius* are of a clonal origin, sharing several characteristics, including a unique plasmid and an identical multilocus sequence type among strains.[120,121] Analysis of genome sequence identified several novel adhesins unique to case clone strains, suggesting these adhesins may play a role in pathogenesis of Brazilian purpuric fever.[122,123]

Identifying the virulence factors that give case clone strains the ability to cause invasive disease will be important in understanding Brazilian purpuric fever. From a broader perspective, characterizing the molecular mechanisms that account for the invasive potential in an otherwise noninvasive bacterium (nontypeable *H. influenzae)* is of great interest in understanding the pathogenesis of invasive bacterial infections.

HAEMOPHILUS DUCREYI

Description of the Pathogen

H. ducreyi is the causative agent of chancroid, an infection characterized by genital ulcer and inguinal lymphadenitis. *H. ducreyi* is a highly fastidious gram-negative coccobacillus. Its microscopic appearance and its nutritional requirement for hemin account for the classification of the bacterium in the genus *Haemophilus.* Despite its name, *H. ducreyi* is not closely related to other members of the genus *Haemophilus* and will likely be reclassified in the future; this issue awaits further study.[124] The organism is a strict human pathogen, and there are no known animal or environmental reservoirs.

Epidemiology

The prevalence of chancroid has declined in the United States, with seven cases reported to the Centers for Disease Control and Prevention (CDC) in 2016. Worldwide, chancroid appears to have declined also, although infection may be occurring is some regions of Africa and the Caribbean.[125,126] However, the clinician must be cautious in interpreting prevalence data because of the difficulty in establishing a diagnosis. Like other genital ulcer disease, chancroid facilitates the transmission of HIV. This association between HIV and chancroid has generated increased interest in understanding the pathogenesis of *H. ducreyi* infection and in improving diagnostic tests for genital ulcer disease. Transmission is primarily heterosexual, and males have outnumbered females in most studies. A high proportion of infected males report sexual contact with commercial sex workers. Chancroid has been strongly associated with illicit drug use. The transmission dynamics of the organism suggest that the disease is likely to be perpetuated in highly sexually active populations, such as commercial sex workers.[127]

H. ducreyi has more recently also been recognized as an important cause of non–sexually transmitted cutaneous ulcers, particularly in the South Pacific and in Africa.[128] Strains that are isolated from cutaneous ulcers have genome sequences that are nearly identical to class I strains (of two related classes) of *H. ducreyi* that cause genital ulcers.[129]

Pathogenesis and Immune Response

Infection occurs as a result of inoculation of bacteria through breaks in the epithelium. Studies using a human model of experimental *H. ducreyi* infection have led to the identification of selected genes or gene clusters that are required for full virulence.[130–132] The bacterium colocalizes with neutrophils and macrophages but is not phagocytized, suggesting that evasion of phagocytic killing is important in pathogenesis. Ulcers contain predominantly T cells, with low numbers of B cells. Patients who have had chancroid may have repeated infections, indicating that natural infection does not confer protective immunity; a similar phenomenon is observed in the human volunteer model.[133]

Clinical Manifestations

The hallmark of chancroid is genital ulceration. The lesion often begins as a papule and evolves into an ulcer. Typical ulcers are painful, well circumscribed with ragged edges, and not indurated. The base of the ulcer

is covered with necrotic material and bleeds easily when scraped. Little or no inflammation of the surrounding skin is present. Approximately half of patients with chancroid have inguinal lymphadenopathy. These lymph nodes sometimes become fluctuant and rupture spontaneously.

Chancroid can manifest in atypical ways. Multiple ulcers may coalesce to form a giant ulcer. Ulceration may resolve before the appearance of inguinal adenopathy and suppuration, resulting in presentation as suppurative inguinal adenitis in the absence of an active genital ulcer. Multiple small ulcers may resemble folliculitis. The main differential diagnostic considerations include primary syphilis (chancre), genital herpes, lymphogranuloma venereum, donovanosis, and condyloma latum of secondary syphilis. In the presence of HIV infection, the number of ulcers at initial presentation may be greater and the duration of ulceration may be longer.

Non–sexually transmitted cutaneous ulcers caused by *H. ducreyi* have clinical features similar to those of yaws, caused by *Treponema pallidum* subsp. *pertenue,* which is endemic where *H. ducreyi* cutaneous ulcers are seen. Ulcers caused by *H. ducreyi* are less likely than yaws to show central granulating tissue and less likely to have indurated edges, but substantial overlap in clinical characteristics exists.

Diagnosis

Because a clinical diagnosis of chancroid is often inaccurate, laboratory confirmation of the diagnosis should be sought. Isolation of *H. ducreyi* from a swab of the lesion or from an aspirate of suppurative lymph nodes confirms the diagnosis. Because the organism is difficult to grow, the use of selective and supplemented media is required. No US Food and Drug Administration (FDA)–approved PCR assay for *H. ducreyi* is available in the United States. Such testing can be performed by clinical laboratories that have developed their own tests and have conducted a Clinical Laboratory Improvement Amendments (CLIA) verification study. However, PCR assay appears more sensitive than culture.[125]

A probable diagnosis of sexually transmitted chancroid for clinical and surveillance purposes can be made according to the following criteria: (1) one or more painful genital ulcers, (2) no evidence of *T. pallidum* infection at darkfield examination of ulcer exudate or with a negative result of a serologic test for syphilis performed at least 7 days after ulcer onset, (3) typical clinical presentation for chancroid, and (4) a negative result of a test for herpes simplex virus on the ulcer exudate.

Therapy

The recommendation of the CDC is a single 1-g dose of azithromycin orally. Alternative regimens include ceftriaxone (250 mg IM in a single dose), ciprofloxacin (500 mg PO twice daily for 3 days), or erythromycin base (500 mg PO three times daily for 7 days). Azithromycin and ceftriaxone have the distinct advantage of single-dose treatment.

Ulcers usually improve symptomatically by 3 days and by objective evaluation by 7 days after initiation of treatment. Lack of improvement should raise several considerations, including coinfection with another pathogen (especially genital herpes or primary syphilis), coexisting HIV infection, which is associated with a delayed response to treatment of chancroid, lack of adherence to the treatment regimen and, finally, resistance of the *H. ducreyi* isolate to the antimicrobial agent prescribed. Isolates from patients who do not respond promptly to treatment should be tested for antimicrobial susceptibility.

Contacts of patients with chancroid should be identified and treated if they had sexual contact with the patient during the 10 days before the onset of symptoms in the patient, even in the absence of clinical symptoms in the contact.

OTHER *HAEMOPHILUS* SPECIES

Description of the Pathogens

Haemophilus species other than *H. influenzae* and *H. ducreyi* are unusual causes of disease in humans. However, as a result of increased awareness of these bacteria as potential pathogens and as a result of improvements in isolating the bacteria in culture, it is now apparent that other *Haemophilus* species more commonly cause human infection than previously believed, particularly in the case of infective endocarditis. *Haemophilus* species are present as part of the normal bacterial flora of the human upper respiratory tract. *Haemophilus parainfluenzae* is the predominant species, accounting for approximately 75% of the *Haemophilus* flora of the human upper airway.

Members of the genus *Haemophilus* are small gram-negative coccobacilli with fastidious growth requirements. The growth requirements are used to distinguish among the species. *Haemophilus* require X factor (hemin), V factor (nicotinamide adenine dinucleotide), or both for growth. These are supplied by erythrocytes, but the erythrocytes must be lysed to release V factor. This growth requirement is supplied in the clinical microbiology laboratory by growing *Haemophilus* species on chocolate agar. Table 225.4 shows differential characteristics of *Haemophilus* and related species that have been documented to cause infection in humans. Species are distinguished based on their different growth requirements for X and V factor, enhancement of growth by carbon dioxide (CO_2), expression of catalase, and ability to cause hemolysis. Real-time PCR assay may be replacing the slower and more onerous identification methods.[2] Species designated as *Aggregatibacter paraphrophilus* (formerly *Haemophilus paraphrophilus*), *H. parainfluenzae,* and *Haemophilus parahaemolyticus* require V factor but not X factor for growth, whereas *Aggregatibacter aphrophilus* and *H. haemolyticus* require X and V, or X only.

Clinical Manifestations

Haemophilus species, particularly *H. parainfluenzae,* and the two species moved from *Haemophilus* to *Aggregatibacter, A. aphrophilus* and *A. paraphrophilus,* are now recognized increasingly as a cause of infective endocarditis, causing up to 5% of cases of endocarditis. *Haemophilus* species are included in the so-called HACEK *(Haemophilus* spp., *Aggregatibacter* spp. [including *Aggregatibacter actinomycetemcomitans*, formerly *Actinobacillus actinomycetemcomitans*], *Cardiobacterium* spp., *Eikenella* spp., and *Kingella* spp.) group of bacteria, which are slow-growing bacteria known to cause endocarditis. The presence of *Haemophilus* species (and others in the HACEK group) should be suspected in patients in whom endocarditis is strongly suspected clinically and who have negative blood cultures. Because these bacteria are slow growing, incubation of blood cultures for 2 weeks has been recommended. However, recent studies indicated that extended incubation does not

TABLE 225.4 Differential Characteristics of *Haemophilus* and *Aggregatibacter* Species

	GROWTH FACTOR REQUIREMENT				
ORGANISM	**X**	**V**	**CO_2 DEPENDENCE**	**HEMOLYSIS**	**CATALASE**
A. aphrophilus	+	–	+	–	–
A. paraphrophilus	–	+	+	–	±
H. parainfluenzae	–	+	–	–	+
H. haemolyticus	+	+	–	±	+
H. parahaemolyticus	–	+	–	+	+
H. ducreyi	+	–	±	±	–

–, Absent; +, present; CO_2, carbon dioxide.

increase recovery from standard automated blood cultures.[134,135] Most patients with endocarditis caused by *Haemophilus* and *Aggregatibacter* species have underlying valvular heart disease. Echocardiography, particularly transesophageal echocardiography, is useful in identifying vegetations and characterizing underlying valvular disease. The clinical course of *Haemophilus* and *Aggregatibacter* endocarditis tends to be subacute, and embolization is common.[136,137]

Other *Haemophilus* species are relatively rare human pathogens, presumably because of their low pathogenic potential. They have been documented as rare causes of a variety of local upper respiratory and systemic infections, including sinusitis, otitis media, conjunctivitis, dental abscess, lower respiratory tract infection, peritonitis, biliary tract infection, brain abscess, osteomyelitis, and wound infections. Most of these are documented in small series and case reports.

Therapy

Treatment should be guided by the antimicrobial susceptibility of the etiologic isolate. The antimicrobial susceptibility characteristics of other *Haemophilus* and related species are similar to those of *H. influenzae,* although fewer data on other *Haemophilus* species are available. Some strains produce β-lactamase and are thus resistant to ampicillin. Agents with generally good activity include trimethoprim-sulfamethoxazole, third-generation cephalosporins, fluoroquinolones, and aztreonam.

In view of the increasing incidence of β-lactamase production among strains of *Haemophilus,* the treatment of choice for *Haemophilus* species endocarditis is now third-generation cephalosporins (ceftriaxone or cefotaxime) or a fluoroquinlone.[138] Treatment of native valve endocarditis should be given for 4 weeks, and treatment for prosthetic valve endocarditis should continue for 6 weeks.

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The complete reference list is available online at Expert Consult.

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