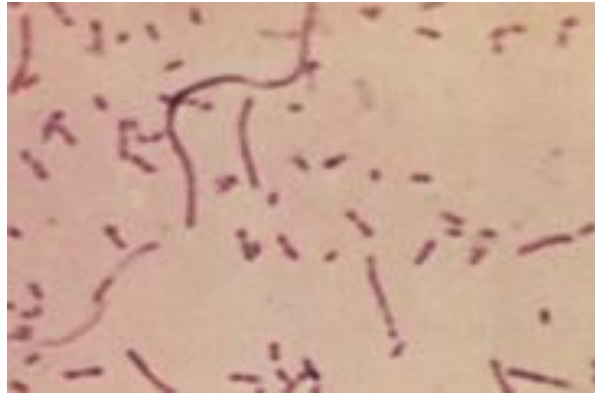


## ***Haemophilus influenza***

### **Introduction**

*Haemophilus influenzae* is a small, nonmotile Gram-negative bacterium in the family *Pasteurellaceae*. The family also includes *Pasteurella* and *Actinobacillus*, two other genera of bacteria that are parasites of animals. Encapsulated strains of *Haemophilus influenzae* isolated from cerebrospinal fluid are coccobacilli, 0.2 to 0.3 to 0.5 to 0.8  $\mu\text{m}$ , similar in morphology to *Bordetella pertussis*, the agent of whooping cough. Non encapsulated organisms from sputum are pleomorphic and often exhibit long threads and filaments. The organism may appear Gram-positive unless the Gram stain procedure is very carefully carried out. Furthermore, elongated forms from sputum may exhibit bipolar staining, leading to an erroneous diagnosis of *Streptococcus pneumoniae*.



Gram stain of *Haemophilus influenzae* from sputum.

*H. influenzae* is highly adapted to its human host. It is present in the nasopharynx of approximately 75 percent of healthy children and adults. It is rarely encountered in the oral cavity, and it has not been detected in any other animal species. It is usually the non-encapsulated strains that are harbored as normal flora, but a minority of healthy individuals (3-7 percent) intermittently harbor *H. influenzae* type b (Hib) encapsulated strains in the upper respiratory tract. Pharyngeal carriage of Hib is important in the transmission of the bacterium. The success of current vaccination programs against Hib is due in part to the effect of vaccination on decreasing carriage of the organism.

## **Characterization of *Haemophilus influenzae***

*Haemophilus influenzae*, formerly called **Pfeiffer's bacillus** or *Bacillus influenzae*, Gram-negative, rod-shaped bacterium first described in 1892 by Richard Pfeiffer during an influenza pandemic. A member of the *Pasteurellaceae* family, it is generally aerobic, but can grow as a facultative anaerobe.<sup>[1]</sup> *H. influenzae* was mistakenly considered to be the cause of influenza until 1933, when the viral etiology of the flu became apparent. Still, *H. influenzae* is responsible for a wide range of clinical diseases.

### **Scientific classification**

Kingdom: Bacteria

Phylum: Proteobacteria

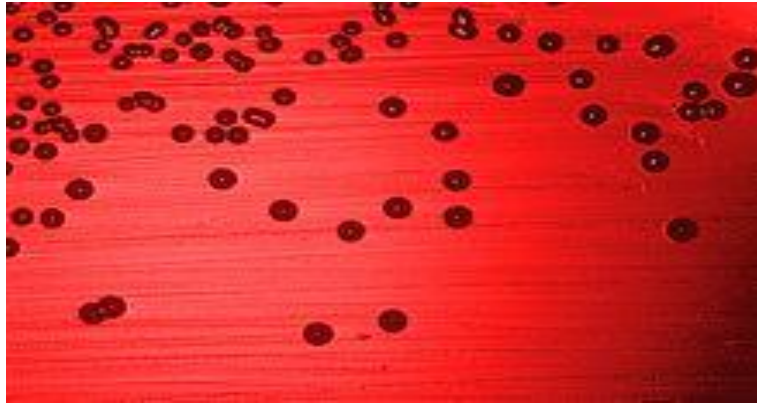
Class: GammaProteobacteria

Order: Pasteurellales

Family: Pasteurellaceae

Genus: *Haemophilus*

Species: *H. influenza*



*H. influenzae* on a blood agar plate.

### **Pathogenesis**

Naturally-acquired disease caused by *H. influenzae* seems to occur in humans only. In infants and young children (under 5 years of age), *H. influenzae* type b causes bacteremia and acute bacterial meningitis. Occasionally, it causes epiglottitis (obstructive laryngitis), cellulitis, osteomyelitis, and joint infections. Nontypable *H. influenzae* causes ear infections (otitis media) and sinusitis in children, and is associated with respiratory tract infections (pneumonia) in infants, children and adults.

Seven serotypes of the bacterium have been identified on the basis of capsular polysaccharides. *H. influenzae* type b is the most important serotype involved in meningitis.

Disease caused by *H. influenzae* usually begins in the upper respiratory tract as nasopharyngitis and may be followed by sinusitis and otitis, possibly leading to pneumonia. In severe cases, bacteremia may occur, which frequently results in joint infections or meningitis.

Infection with *Haemophilus influenzae* type b (Hib) can result in meningitis and other severe infections (e.g., pneumonia, bacteremia, cellulitis, septic arthritis, and epiglottitis) primarily among infants and children <5 years of age. Hib disease is uncommon in individuals 5 years of age or older. Hib meningitis has a case-fatality ratio of 5-10% in the United States even with initiation of early antimicrobial therapy. As a result of the widespread use of Hib conjugate vaccines, the disease is now uncommon in the U.S. and is seen primarily in infants too young to be vaccinated and unvaccinated children. The pathogenesis of *H. influenzae* infections is not completely understood, although the presence of the type b polysaccharide capsule is known to be the major factor in virulence. Encapsulated organisms can penetrate the

epithelium of the nasopharynx and invade the blood capillaries directly. Their capsule allows them to resist phagocytosis and complement-mediated lysis in the nonimmune host. Nontypable (non-encapsulated) strains are less invasive, but they are apparently able to induce an inflammatory response that causes disease. Outbreaks of *H. influenzae* type b infection may occur in nurseries and child care centers, and prophylactic administration of antibiotics is warranted. Vaccination with type b polysaccharide (in the form of Hib conjugate vaccines) is effective in preventing infection, and several vaccines are now available for routine use.

### **Transmission of *Haemophilus influenzae***

The *H. influenzae* bacteria live in the upper respiratory tract and are usually transmitted by close contact with an infected individual. Droplets in the air from a sneeze can be inhaled and may also cause infection.

### **Virulence factors**

*H. influenzae* does not produce any demonstrable exotoxins. The direct role of endotoxin in meningitis or bacteremia is unclear, although the Gram-negative

bacterium's outer membrane lipooligosaccharide (LOS) is thought to play a role in inflammation associated with otitis media. All virulent strains produce neuraminidase and an IgA protease, but the role of these extracellular enzymes in invasion is unproven. Fimbriae increase the adherence of bacteria to human mucosal cells *in vitro*, and they are required for successful colonization of the nasopharynx. The Anton antigen, as defined in red blood cells, appears to be the receptor.

Virulence, at least in the case of bacteremia and meningitis, is directly related to capsule formation. Virtually all of these infections are caused by the type b serotype, and its capsular polysaccharide, containing ribose, ribitol and phosphate, is the proven determinant of virulence. The capsule material is antiphagocytic, and it is ineffective in inducing the alternative complement pathway, so that the bacterium can invade the blood or cerebrospinal fluid without attracting phagocytes or provoking an inflammatory response and complement-mediated bacteriolysis. For this reason, anticapsular antibody, which promotes both phagocytosis and lysis of

bacteria, is the main factor in immune defense against *H. influenzae* infections.

The polyribosylribitol phosphate (PRP) capsule is the most important virulence factor because it renders type b *H. influenzae* resistant to phagocytosis by polymorphonuclear leukocytes in the absence of specific anticapsular antibody, and it reduces the bacterium's susceptibility to the bactericidal effect of serum. However, susceptibility to the bactericidal effect of serum depends on the presence of antibodies to a number of other antigenic sites, including the lipooligosaccharide and outer membrane proteins designated as P1 and P2.

Type b *H. influenzae* is plainly the most virulent of the *Haemophilus* species; 95 percent of bloodstream and meningeal *Haemophilus* infections in children are due to this bacterium. In contrast, in adults, nontypable strains of *H. influenzae* are the most common cause of *Haemophilus* infection, presumably because most adults have naturally acquired antibody to PRP.



## **Serotypes**

In 1930, two major categories of *H. influenzae* were defined: the unencapsulated strains and the encapsulated strains. Encapsulated strains were classified on the basis of their distinct capsular antigens. There are six generally recognized types of encapsulated *H. influenzae*: a, b, c, d, e, and f.<sup>[2]</sup> Genetic diversity among unencapsulated strains is greater than within the encapsulated group. Unencapsulated strains are termed nontypable (NTHi) because they lack capsular serotypes; however, they can be classified by multilocus sequence typing. The pathogenesis of *H. influenzae* infections is not completely understood, although the presence of the capsule in encapsulated type b (Hib), a serotype causing conditions such as epiglottitis, is known to be a major factor in virulence. Their capsule allows them to resist phagocytosis and complement-mediated lysis in the nonimmune host. The unencapsulated strains are almost always less invasive; they can, however, produce an inflammatory response in humans, which can lead to many symptoms. Vaccination with Hib conjugate vaccine is effective in preventing Hib infection. Several

vaccines are now available for routine use against Hib, but vaccines are not yet available against NTHi.

## **Diagnosis**

Clinical diagnosis of *H. influenzae* is typically performed by bacterial culture or latex particle agglutinations. Diagnosis is considered confirmed when the organism is isolated from a sterile body site. In this respect, *H. influenzae* cultured from the nasopharyngeal cavity or sputum would not indicate *H. influenzae* disease, because these sites are colonized in disease-free individuals.<sup>[4]</sup> However, *H. influenzae* isolated from cerebrospinal fluid or blood would indicate *H. influenzae* infection.

## **Culture**

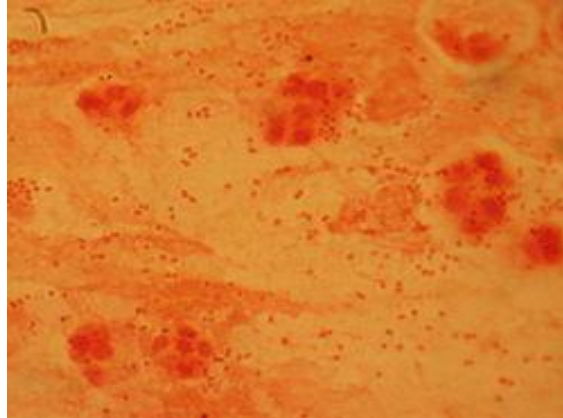
Bacterial culture of *H. influenzae* is performed on agar plates, the preferable one being chocolate agar, with added X(hemin) & V(NAD) factors at 37°C in a CO<sub>2</sub>-enriched incubator.<sup>[5]</sup> Blood agar growth is only achieved as a satellite phenomenon around other bacteria. Colonies of *H. influenzae* appear as convex, smooth, pale, grey or transparent colonies. Gram-stained and microscopic observation of a specimen of *H. influenzae*

will show Gram-negative, coccobacilli, with no specific arrangement. The cultured organism can be further characterized using catalase and oxidase tests, both of which should be positive. Further serological testing is necessary to distinguish the capsular polysaccharide and differentiate between *H. influenzae* b and nonencapsulated species.

Although highly specific, bacterial culture of *H. influenzae* lacks in sensitivity. Use of antibiotics prior to sample collection greatly reduces the isolation rate by killing the bacteria before identification is possible.<sup>[6]</sup> Beyond this, *H. influenzae* is a finicky bacterium to culture, and any modification of culture procedures can greatly reduce isolation rates. Poor quality of laboratories in developing countries has resulted in poor isolation rates of *H. influenzae*.

*H. influenzae* will grow in the hemolytic zone of *Staphylococcus aureus* on blood agar plates; the hemolysis of cells by *S. aureus* releases factor V which is needed for its growth. *H. influenzae* will not grow outside the hemolytic zone of *S. aureus* due to the lack of nutrients such as factor V in these areas. Fildes agar is

best for isolation. In Levinthal medium capsulated strains show distinctive iridescence.



*H. influenzae*, in a Gram stain of a sputum sample, appear as Gram-negative coccobacilli.<sup>[3]</sup>



*Haemophilus influenzae* requires X and V factors for growth. In this culture haemophilus has only grown around the paper disc that has been impregnated with X and V factors. There is no bacterial growth around the discs that only contain either X or V factor.

## **Latex particle agglutination**

The latex particle agglutination test (LAT) is a more sensitive method to detect *H. influenzae* than culture.<sup>[7]</sup>

Because the method relies on antigen rather than viable bacteria, the results are not disrupted by prior antibiotic use. It also has the added benefit of being much quicker than culture methods. However, antibiotic sensitivity is not possible with LAT, so a parallel culture is necessary.

## **Molecular methods**

Polymerase chain reaction (PCR) assays have been proven to be more sensitive than either LAT or culture tests, and highly specific.<sup>[7]</sup> However, PCR assays have not yet become routine in clinical settings. Countercurrent immunoelectrophoresis has been shown to be an effective research diagnostic method, but has been largely supplanted by PCR.

## **Treatment**

*Haemophilus influenzae* produces beta-lactamases, and it is also able to modify its penicillin-binding proteins, so it has gained resistance to the penicillin family of antibiotics. In severe cases, cefotaxime and ceftriaxone

delivered directly into the bloodstream are the elected antibiotics, and, for the less severe cases, an association of ampicillin and sulbactam, cephalosporins of the second and third generation, or fluoroquinolones are preferred. (Fluoroquinolone-resistant *Haemophilus influenzae* has been observed.)<sup>[8]</sup>

Macrolide antibiotics (e.g., clarithromycin) may be used in patients with a history of allergy to beta-lactam antibiotics. Macrolide resistance has also been observed.<sup>[9]</sup>

## **Prevention**

Effective vaccines for *Haemophilus influenzae* have been available since the early 1990s, so it is preventable. The World Health Organization recommends a pentavalent vaccine, combining vaccines against diphtheria, tetanus, pertussis, hepatitis B and Hib. There is not yet sufficient evidence on how effective this pentavalent vaccine is in relation to the individual vaccines.<sup>[10]</sup> Hib vaccines cost about seven times the total cost of vaccines against measles, polio, tuberculosis, diphtheria, tetanus, and pertussis. Consequently, whereas 92% of the populations

of developed countries was vaccinated against Hib as of 2003, vaccination coverage was 42% for developing countries, and only 8% for least-developed countries.<sup>[11]</sup>

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