Haemophilus and related bacteria

- Three most important genera in the family Pasteurellaceae are *Haemophilus*, *Actinobacillus*, and *Pasteurella*.
- Small, gram-negative, non-spore-forming, nonmotile, and aerobic or facultative anaerobic rods.
- Most have fastidious growth needs (enriched media for isolation).
- Members of the genus *Haemophilus* are the most commonly isolated
Haemophilus spp and related infections

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Haemophilus

- Haemophilae are small, pleomorphic, gram-negative rods present on the mucous membranes of humans.

- *Haemophilus influenzae* is the species most commonly associated with disease, with infections most often reported in pediatric patients before the introduction of the *H. influenzae* type b (HIB) vaccine.
**Haemophilus aegyptius**

- An important cause of acute, purulent conjunctivitis.
- Taxonomically, *H. aegyptius* belongs in the species *H. influenzae* but has traditionally been separated.

*H. influenzae* biogroup *aegyptius* is phenotypically related to *H. aegyptius* but is taxonomically distinct.

- Biogroup *aegyptius* is the etiologic agent of the fulminant pediatric disease *Brazilian purpuric fever*. 
**Haemophilus**

- *Haemophilus ducreyi*: the etiologic agent of the sexually transmitted disease **soft chancre**, or chancroid.
- Genomic analysis: *H. ducreyi* is a member of the family Pasteurellaceae but does not belong in the genus *Haemophilus*.
- *Haemophilus aphrophilus* is an uncommon but important cause of endocarditis.
- *Haemophilus parainfluenzae* is the most common species in the mouth but are rarely pathogenic, being responsible primarily for opportunistic infections.
Media supplemented with the following growth-stimulating factors:

- (1) **hemin** (also called **X factor** for unknown factor)
- (2) **nicotinamide adenine dinucleotide** (NAD; also called **V factor** for "vitamin")
- (3) both

Heated blood ("chocolate") agar is used for the in vitro isolation of *Haemophilus*. 
Cell wall structure

- Typical of other gram-negative rods.

- Lipopolysaccharide with endotoxin activity is present in the cell wall.

- The surface of many but not all strains of *H. influenzae* is covered with a polysaccharide capsule, and six antigenic serotypes (a through f) have been identified.
Polysaccharide capsule

- Before the introduction of the HIB vaccine, *H. influenzae* serotype b was responsible for more than 95% of all invasive *Haemophilus* infections.
- After the introduction of the vaccine, most disease caused by this serotype disappeared.
- Currently, serotypes c and f and nonencapsulated *H. influenzae* are responsible for most *H. influenzae* disease.
Biogroups - Biotypes

- *H. influenzae* is subdivided into eight biotypes (I through VIII) as determined by three biochemical reactions: indole production, urease activity, and ornithine decarboxylase activity.

- Subdivided into biogroups, which is useful for clinical purposes. *H. influenzae* biogroup *aegypticus* is important because it causes Brazilian purpuric fever.
**H. parainfluenzae and nonencapsulated H. influenzae**

- Colonize the upper respiratory tract in virtually all people within the first few months of life.

- Can spread locally and cause disease in the ears (otitis media), sinuses (sinusitis), and lower respiratory tract (bronchitis, pneumonia). Disseminated disease is relatively uncommon.

- Encapsulated *H. influenzae* (particularly serotype b [biotype I]) is a common cause of disease in unvaccinated children.
Colonization

- Pili and nonpilus adhesins mediate colonization of the oropharynx

- Cell wall components of the bacteria (e.g., lipopolysaccharide) impair ciliary function

- In the absence of specific opsonic antibodies high-grade bacteremia can develop, with dissemination to the meninges or other distal foci.
Antiphagocytic polysaccharide capsule

- Contains ribose, ribitol, and phosphate (commonly referred to as polyribitol phosphate [PRP]).

- Antibodies develop as a result of natural infection, vaccination with purified PRP, or the passive transfer of maternal antibodies.

- Immunoglobulin (Ig)A1 proteases: facilitate colonization of the organisms on mucosal surfaces.
Epidemiology

- Noncapsular *Haemophilus* commonly colonized in humans;

- Disease caused by *H. influenzae* type b was primarily a pediatric problem; eliminated in immunized populations

- *H. influenzae* type b is the most common serotype that causes systemic disease
Epidemiology - *H. ducreyi*

- Important cause of genital ulcers (chancroid) in Africa and Asia but is less common in Europe and North America.

- With the exception of *H. ducreyi*, which is spread by sexual contact, most *Haemophilus* infections are caused by the patient's bacterial flora (endogenous infections)
H. influenzae type b was the most common cause of pediatric meningitis.

This situation changed rapidly when the conjugated vaccines became widely used.

A disease primarily of unimmunized children characterized by fever, severe headache, and systemic signs.
Meningitis

- 1- to 3-day history of mild upper respiratory disease
- After which the typical signs and symptoms of meningitis appear.
- Mortality is less than 10% in patients who receive prompt therapy, a low incidence of serious neurologic sequelae
- Person-to-person spread in a nonimmune population is well documented, so appropriate epidemiologic precautions must be used.
Epiglottitis

- The peak incidence during the prevaccine era occurred in children 2 to 4 years of age

- Fever, and difficulty breathing, which can progress rapidly to obstruction of the airway and death.
Cellulitis

- A pediatric disease that has largely been eliminated by vaccination.
- Fever and cellulitis (reddish-blue patches on the cheeks or periorbital areas).
- Diagnosis suggested by the typical clinical presentation (cellulitis proximal to the oral mucosa, and lack of documented vaccination in the child).
Arthritis

- Before the advent of conjugated vaccines, the most common form of arthritis in children younger than 2 years
- Single, large joint secondary to the bacteremic spread of *H. influenzae* type b.
- Immunocompromised patients and patients with previously damaged joints.
Otitis, Sinusitis, and Lower Respiratory Tract Disease

- Nonencapsulated strains (primarily biotypes II and III)

- *H. influenzae* and *Streptococcus pneumoniae* are the two most common causes of acute and chronic otitis and sinusitis.

- Colonize patients who have chronic pulmonary disease (including cystic fibrosis), and are associated with exacerbation of bronchitis and pneumonia.
Conjunctivitis

- *H. aegyptius*, also called the Koch-Weeks bacillus
- an acute, purulent conjunctivitis.
- Associated with epidemics, particularly during the warm months of the year.

Brazilian Purpuric Fever

- *H. influenzae* biogroup *aegyptius* (an organism that is different from *H. aegyptius*)
- An initial conjunctivitis, fever, vomiting, and abdominal pain.
- Petechiae, purpura, and shock culminating in death
- Pathogenesis poorly understood.
Chancroid

- A sexually transmitted disease
- Most commonly diagnosed in men
- Women can have asymptomatic or inapparent disease
- 5 to 7 days after exposure, a tender papule with an erythematous base develops on the genital area
- Within 2 days the lesion ulcerates and becomes painful, and inguinal lymphadenopathy is commonly present.
Other species of *Haemophilus*

- Cause opportunistic infections, such as otitis media, conjunctivitis, sinusitis, meningitis, and dental abscesses.

- Some species that stick to tooth surfaces, such as *H. aphrophilus*, can spread from the mouth to the blood and then stick to a previously damaged heart valve or artificial valve, leading to subacute endocarditis.
LABORATORY DIAGNOSIS
Specimen Collection and Transport

- Meningitis: obtain samples of cerebrospinal fluid (CSF) and blood to diagnose *Haemophilus* meningitis.

- Approximately $10^7$ bacteria per ml of CSF in patients with untreated meningitis, 1 to 2 ml of fluid is generally adequate for microscopy, culture, and antigen-detection tests.
Specimen Collection and Transport

- Epiglottitis, cellulitis, arthritis, or pneumonia: blood cultures should be performed.

- Direct needle aspiration should be performed for the microbiologic diagnosis of sinusitis or otitis.

- Specimens should not be collected from the posterior pharynx in patients with suspected epiglottitis, because the procedure may stimulate coughing and obstruct the airway.
Specimen Collection - *H. ducreyi*

- Specimens for the detection of *H. ducreyi* should be collected with a moistened swab from the base or margin of the ulcer.

- Cultures of pus collected by aspiration from an enlarged lymph node can be performed but is generally less sensitive than culture of the ulcer.

- The laboratory should be notified that *H. ducreyi* is suspected, because special culture techniques must be used.
Microscopy

- Is both sensitive and specific
- Gram-negative rods ranging in shape from coccobacilli to long, pleomorphic filaments can be detected in more than 80% of CSF specimens from patients with untreated Haemophilus meningitis
- Is also useful for the rapid diagnosis of the organism in arthritis and lower respiratory tract disease.
Culture

- Inoculated onto media supplemented with appropriate growth factors: chocolate agar or Levinthal's agar
- If chocolate agar is overheated during preparation, V factor is destroyed; *Haemophilus* species requiring this growth factor (e.g., *H. influenzae*, *H. aegyptius*, *H. parainfluenzae*) will not grow.
- 1- to 2-mm, smooth, opaque colonies after 24 hours of incubation.
Satellite phenomenon

- Grow around colonies of *Staphylococcus aureus* on unheated blood agar (*satellite phenomenon*).

- The staphylococci provide the requisite growth factors by lysing the erythrocytes in the medium and releasing intracellular heme (X factor) and excreting NAD (V factor).
Blood cultures

- Are generally delayed, because most commercially prepared blood culture broths are not supplemented with optimum concentrations of X and V factors.
**H. aegyptius and H. ducreyi cultures**

- *H. aegyptius* grows on chocolate agar supplemented with 1% IsoVitaleX with growth detected after incubation in a carbon dioxide atmosphere for 2 to 4 days.

- *H. ducreyi*: on gonococcal (GC) agar supplemented with 1% to 2% hemoglobin, 5% fetal bovine serum, IsoVitaleX enrichment, and vancomycin (3 μg/ml). Cultures should be incubated at 33°C in 5% to 10% carbon dioxide for 7 days or more.
Antigen Detection

- Detection of the PRP capsular antigen
- Particle agglutination: antibody-coated latex particles are mixed with the clinical specimen; agglutination occurs if PRP is present.
- Detected in CSF and urine (in which the antigen is eliminated intact).
- Has limited use because it can detect only *H. influenzae* type b.
Identification - *H. influenzae*

- Identified by the demonstration of a requirement for both X and V factors and the specific biochemical properties.
TREATMENT

- **Systemic** *H. influenzae* infections require prompt antimicrobial therapy.
- Broad-spectrum cephalosporins.
- Less severe infections such as sinusitis and otitis can be treated with ampicillin (if susceptible, approximately 30% of strains are resistant), cephalosporin, azithromycin, or a fluoroquinolone.
- *H. ducreyi* are susceptible to erythromycin
PREVENTION AND CONTROL

- **Active immunization** with purified capsular PRP

- Three doses of vaccine against *H. influenzae* type b disease before the age of 6 months, followed by booster doses.

- Antibiotic **chemoprophylaxis** is used to eliminate the carriage of *H. influenzae* type b in children at high risk for disease.

- Rifampin prophylaxis has been used in these settings.