

A stack of books is shown from a low angle, with the spines of several books visible. The books have various colors, including red, orange, and yellow. A semi-transparent gradient overlay in shades of red and orange is positioned over the books. The word "HAEMOPHILUS" is written in white, bold, uppercase letters on the red part of the overlay.

HAEMOPHILUS

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Learning objectives

At the end of the session, the students will be able to

- Describe morphology and antigens
- Describe Pathogenesis & Clinical features
- Choose appropriate lab diagnosis and interpret the results
- Describe prevention and treatment



General Features

- Pleomorphic gram-negative bacilli
- Require special growth factors present in blood (factor X and V)
- oxidase positive
- Capsulated
- **Important Species:**
 - *H. influenzae*
 - *H. ducreyi*
 - *H. aegyptius*
 - *H. haemolyticus*
 - *H. parahaemolyticus*
 - *H. parainfluenzae*



HAEMOPHILUS INFLUENZAE

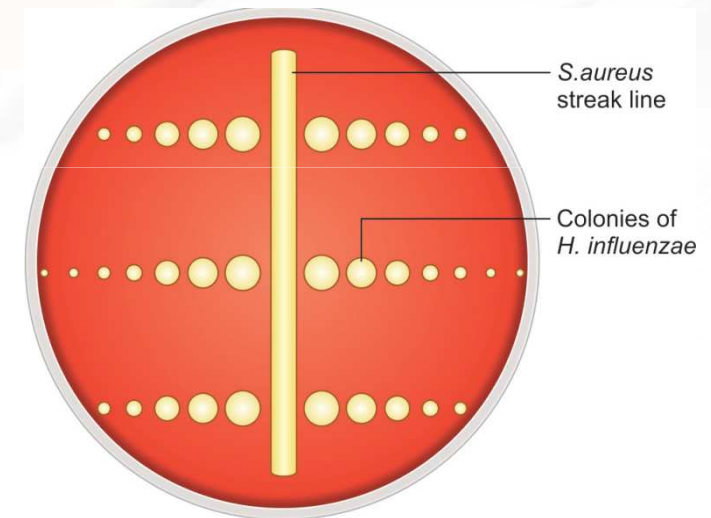


HAEMOPHILUS INFLUENZAE

- **Growth Requirements**
- **Factor X** - hemin or other porphyrins required for the synthesis of enzymes such as cytochrome, catalase and peroxidase; involved in the aerobic respiration
- **Factor V** - nicotinamide adenine dinucleotide (NAD)
- **Does not grow on ordinary media**

Growth Requirements

- **Growth is scanty on blood agar**
 - Only factor X is available in this medium and V factor is present mainly inside the RBCs
 - Sheep blood contains NADase that destroys factor V
- **Grows well on chocolate agar**
- **Satellitism** - *S. aureus* is streaked across a blood agar





Virulence Factors

- **Capsular polysaccharide** - most important – inhibits phagocytosis
- **Endotoxin** - inflammatory response
- **Outer member proteins**
- **IgA 1 proteases** - inactivate IgA 1 present on the mucosal surface
- **Pili & other adhesion proteins** - help in colonization on epithelial surface



Clinical Manifestations of Hib

- **Central nervous system infections:**
- **Pyogenic meningitis:**
 - Children < 2 yrs - fever, neck rigidity, vomiting, headache and altered sensorium
- **Subdural effusion:**
 - Common complication following meningitis - seizures or hemiparesis
 - Mortality rate high. Neurologic sequelae
- **Epiglottitis: Life threatening**
 - Affects older children (2–7 years old) & rarely adults



Clinical Manifestations of Hib

- **Pneumonia in infants:**
 - Similar to other bacterial pneumonia with more pleural involvement
- **Less common invasive conditions seen in children**
 - Cellulitis of neck and head region
 - Osteomyelitis, septic arthritis
 - Pericarditis
 - Orbital cellulitis, endophthalmitis
 - Urinary tract infection
 - Bacteremia without an identifiable focus.



Nontypeable H. influenzae

- Contagious spread and usually affect adults
 - Childhood otitis media
 - Exacerbations of COPD – MC cause
 - Pneumonia in adults with underlying COPD or AIDS
 - Puerperal sepsis and neonatal bacteremia
 - Sinusitis in adults and children
 - Invasive infections - rarely

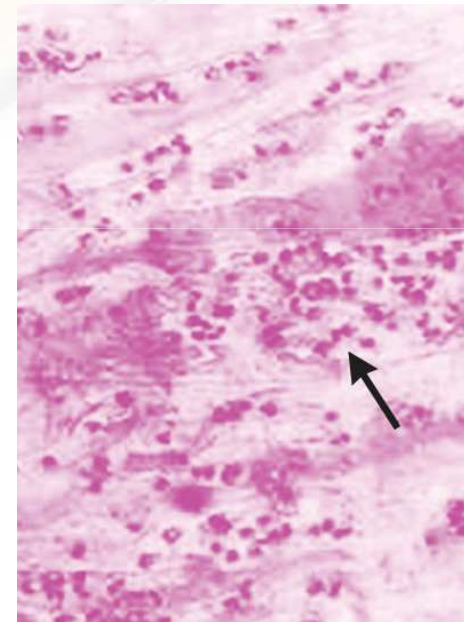
A background image showing a laboratory setting with a person's hands holding a white container, possibly a test tube or pipette tip, over a surface. The image is overlaid with a semi-transparent orange and red gradient.

Laboratory Diagnosis

- **Specimen Collection and Transport**
- cerebrospinal fluid (CSF), blood, sputum, pus, aspirates from joints, middle ears or sinuses
- Highly sensitive to low temperature - specimens **should never be refrigerated**

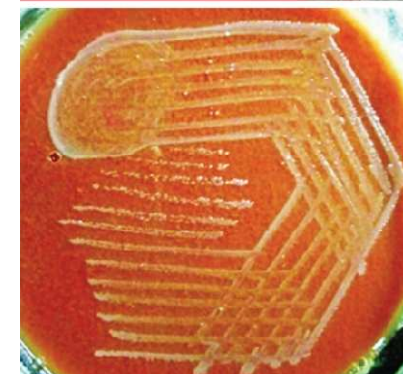
Direct Detection

- **Gram staining of specimen** - pleomorphic gram-negative coccobacilli
- **Capsule detection (Quellung reaction)**
- **Antigen detection: The type b capsular antigen** in CSF, urine or other body fluids - latex agglutination test or direct-IF test



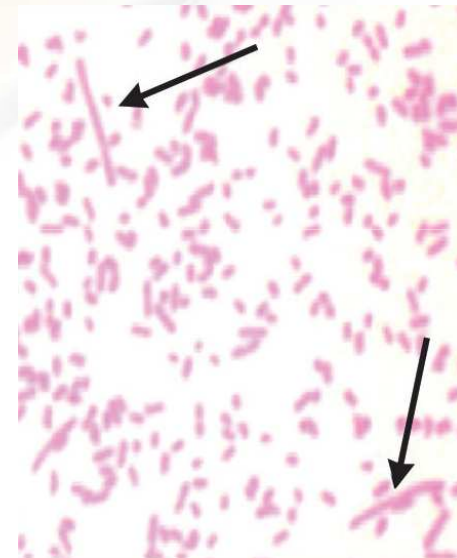
Culture

- **Highly fastidious**, requires the **factor X and V**, enhanced by 5–10% CO₂
- **Blood agar** with *S. aureus* streak line: **satellitism**
- Chocolate agar: grows well
- **Fildes agar and Levinthal's agar** –
Transparent media - iridescent colonies
- **Haemophilus selective medium**: contains bacitracin & sucrose



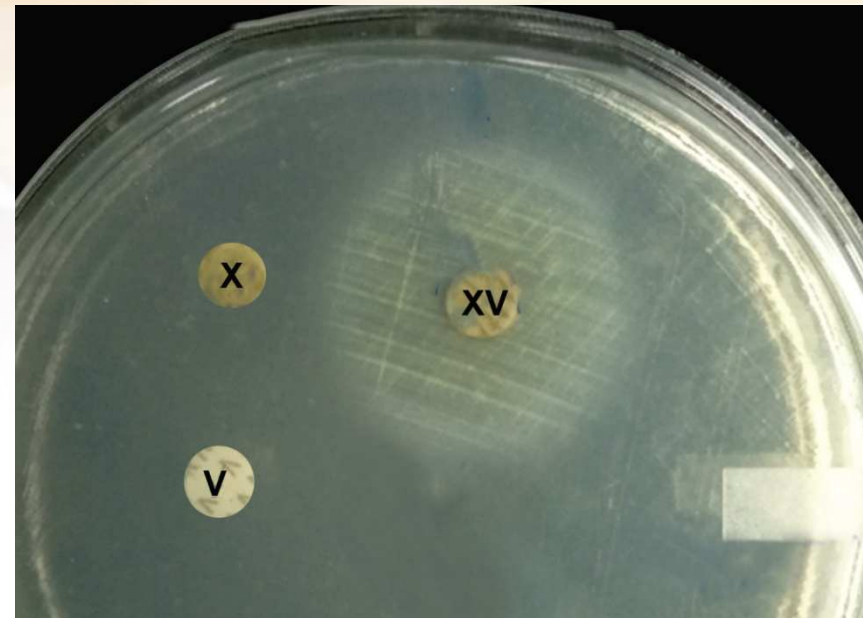
Lab Diagnosis

- **Culture Smear**
 - **Gram staining** - reveals pleomorphic gram-negative nonmotile bacilli
- **Biochemical Tests**
 - Catalase positive and oxidase positive
 - Reduces nitrate to nitrite
 - Ferments glucose & xylose but not sucrose, lactose & mannitol



Lab diagnosis

- **Disk Test for X and V**
Requirement: *Haemophilus*
- species vary in their X and V requirement.
- Growth surrounding X & XV discs only: *H. Ducreyi* and *A. aphrophilus*
- Growth surrounding V and XV discs only: *H. parainfluenzae*, *H. parahaemolyticus* and *A. paraphrophilus*



Growth characteristics of various *Haemophilus* species

Haemophilus species	Growth requirements			Hemolysis on blood agar**
	X	V	CO ₂	
<i>H.influenzae</i>	+	+	-	-
<i>H.aegyptius</i>	+	+	-	-
<i>H.haemolyticus</i>	+	+	-	+
<i>H.ducreyi</i>	+	-	V*	V*
<i>H.aphrophilus</i>	+	-	+	-
<i>H.parainfluenzae</i>	-	+	-	-
<i>V.parahaemolyticus</i>	-	+	-	+
<i>H.paraphrophilus</i>	-	+	+	-

Differences between type b and nontypeable *Haemophilus* strains

	Type b strains	Non typeable strains
Capsule	Made up of poly ribosyl ribitol phosphate (PRP)	Non Capsulated
Manifestations	Invasive- meningitis, epiglottitis, pneumonia, bacteremia, endocarditis	Non-invasive – otitis media (in children) and pneumonia (adult)
Age	Affect children	Affect adult
Spread	Hematogenous spread	Contiguous spread
Vaccine	Hib vaccine available	Not available



Typing Methods

- **Biotyping**
 - **Based on three biochemical** properties (indole, ornithine decarboxylase and urease)
 - *H. influenzae* has eight biotypes (I–VIII)
 - Most clinical isolates belong to type I, II and III
 - Majority of invasive type b strains belong to biotype I
- **Serotyping:** using type-specific antisera.



Treatment

- **Invasive infections Hib**
 - Cephalosporins (ceftriaxone, cefotaxime) - DOC
 - Nontypeable strains of *H. influenzae* are often *resistant* to β lactams
 - β -lactamase production (20–35% of strains)
 - Expressing altered penicillin binding protein



Prophylaxis

- **Hib Conjugate Vaccine**
 - PRP capsular antigen of Hib
 - Capsular antigens are poorly immunogenic to children - conjugated with adjuvants (diphtheria toxoid, tetanus toxoid & *N. Meningitidis*)
- Can also reduce the rate of pharyngeal colonization with Hib
- **Schedule:** Under national immunization program, Hib vaccine is given in combination with DPT, hepatitis B (pentavalent vaccine) at 6, 10 and 14 weeks of birth
- Route - IM, at anterolateral side of mid thigh



HAEMOPHILUS DUCREYI

Haemophilus ducreyi

- **Chancroid (or soft chancre) - sexually transmitted infection**
 - Painful genital ulceration that bleeds easily
 - No inflammation of surrounding skin
 - Enlarged, tender inguinal lymph nodes (bubo)





Haemophilus ducreyi

- **Epidemiology**
 - Common cause of genital ulcers in developing countries.
 - Transmission predominantly heterosexual
 - Males to females ratio - 3:1 to 25:1
 - Chancroid and HIV:
- *Laboratory Diagnosis*
- **Specimens:** Exudate or swab from the edge of the ulcer and lymph node aspirate
- **Direct microscopy:**
 - **Pleomorphic gram-negative coccobacillus;** occurs in groups or in parallel chains
 - Bipolar staining
 - **School of fish or rail road track appearance.**



Haemophilus ducreyi

- **Culture:**
 - Requires factor X (hemin), but not factor V
 - Rabbit blood agar/chocolate agar enriched with 1% isovitalex and made selective by adding vancomycin
 - Chorioallantoic membrane of the chick embryo
- **Optimum conditions** - 10% CO₂, high humidity & incubation at 35°C for 2–8 days
- **Biochemical reactions:** *biochemically inert*



Haemophilus ducreyi

- **Slide agglutination test:** specific antiserum confirmative
- **Multiplex PCR assay** - *H. ducreyi*, *Treponema pallidum* & *herpes simplex virus*.
- **Treatment**
 - Azithromycin – DOC
 - Alternative drugs: Ceftriaxone, ciprofloxacin or Erythromycin



OTHER HAEMOPHILUS SPECIES



Haemophilus aegyptius

- **Koch-Weeks bacillus;**
- **Closely resembles *H. influenzae* biotype III** except more predilection for conjunctiva and not occurring as pharyngeal carrier
- Purulent contagious conjunctivitis (Egyptian ophthalmia)
- **Brazilian purpuric fever:** Fever, purpura, hypotension & shock
- Requires both factors X and V, similar to *H. influenzae*, but differs by—
 - Fails to ferment xylose
 - Hemagglutination with guinea pig RBC at 4°C
 - Slower growth than *H. influenzae*.



Haemophilus parainfluenzae

- Commensal in mouth and throat
- Opportunistic pathogen - endocarditis, conjunctivitis, abscesses, genital tract infections & bronchopulmonary infections in patients with cystic fibrosis
- It differs from *H. influenzae* by:
 - Requires only factor V, but not X
 - Ferments sucrose, but not xylose.



H. haemolyticus & *H. parahaemolyticus*

- Commensals in oropharynx
- Differ from *H. influenzae* in being β hemolytic, best produced in sheep or ox blood agar & when incubated aerobically
- *H. parahaemolyticus* - rare cause of endocarditis
- *H. haemolyticus* requires both factors X and V, whereas *H. parahaemolyticus* requires only factor V



Aggregatibacter aphrophilus & *A. paraphrophilus*

- Capnophilic & require 5–10% of CO₂ for optimum growth.
- *A.aphrophilus* requires only factor X, whereas *A.paraphrophilus* requires only factor V
- Commensals of mouth and occasionally cause endocarditis, head & neck infections, invasive bone and joint infections



HACEK GROUP



HACEK

- Group of highly fastidious, slow-growing, capnophilic, gram negative bacteria
 - Normally oral commensal
 - Occasionally local infections of mouth & bacterial endocarditis
- ***Haemophilus parainfluenzae***
 - ***Aggregatibacter actinomycetemcomitans***
 - *A. aphrophilus* & *A. paraphrophilus*
 - ***Cardiobacterium hominis***
 - ***Eikenella corrodens***
 - ***Kingella kingae***



HACEK ENDOCARDITIS

- 3% of total endocarditis cases
- Subacute course
- Patients with preexisting valvular defects or those undergoing dental procedures
- Aortic & mitral valves - most commonly affected



Laboratory Diagnosis

- Highly fastidious, require special media
- Capnophilic - 5–10% of CO₂
- Incubation up to 30 days – detection time reduced to 1 week if automated culture systems (BacT/ALERT) used
- PCR – more sensitive and rapid than culture



Aggregatibacter actinomycetemcomitans

- Most common member of HACEK to cause endocarditis.
- Soft tissue infections & abscesses, periodontitis, brain abscess, meningitis & endophthalmitis
- **Laboratory diagnosis:**
 - Small non-motile gram-negative coccobacillus
 - Grows on blood agar supplemented with 5% defibrinated horse blood
 - Catalase and nitrate positive
 - Ferments only glucose, galactose and maltose
 - Negative for indole, citrate, urease and decarboxylase tests.



Cardiobacterium hominis

- Frequently affects the aortic valve
- Also associated with arterial embolization, immune complex glomerulonephritis or arthritis.
- **Laboratory diagnosis:**
 - Non-motile, non-capsulated pleomorphic & gram-negative bacillus
 - Grows on blood agar under 3–5% CO₂ and high humidity
 - Ferments a wide range of sugars and forms indole
 - Oxidase positive, but catalase and nitrate negative.

A photograph of a laboratory setting. In the foreground, a white petri dish is partially visible, containing a light-colored agar medium. A pipette tip is positioned above the dish, as if about to dispense liquid. The background is slightly blurred, showing a red surface, possibly a lab bench or equipment. The overall lighting is bright and clinical.

Eikenella corrodens

- Small slender non-capsulated gram-negative bacillus
- Endocarditis, skin and soft tissue infections
- **Laboratory diagnosis:**
 - Lacks flagella, **twitching or jerky motility** due to contraction of fimbria
 - 'Corrodens' - **pitting or corroded colonies on blood agar**
 - Oxidase-positive, catalase negative
 - Positive for lysine and ornithine decarboxylase tests
 - Indole, citrate and urease tests: negative



Kingella kingae

- Infections of bones, joints and tendons
- Non-motile, and gram-negative
- Exists as as coccobacillary and diplococcal forms
- Oxidase-positive, catalase negative