

Haemophilus



Jagrity singh (MSC 2nd sem)

Scientific classification

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma Proteobacteria

Order: Pasteurellales

Family: Pasteurellaceae

Genus: Haemophilus

Species: influenzae

Binomial name: *Haemophilus influenzae*

History

- Pfeiffer 1892 Mistaken as causative of Human Influenza
- Isolated by Smith, Andrewes and Laidlaw in 1933
- Need one or both of Accessory growth factors X and V present in the Blood

Overview- Haemophilus

- Small
- Non-motile
- Gram-negative rods
- Transmitted via respiratory droplets, or direct contact with contaminated secretions
- Normal flora of the human respiratory tract and oral cavity.

Haemophilus species of clinical importance

1. *H. influenzae*

-type b is an important human pathogen

2. *H. ducreyi*

-sexually transmitted pathogen (chancroid)

3. Other *Haemophilus* are normal flora

- *H. parainfluenzae* – Pneumonia & endocarditis

- *H. aphrophilus* – Pneumonia & endocarditis

- *H. aegyptius* – Pink eye (purulent conjunctivitis)

Haemophilus Influenza

- Aerobic gram-negative bacteria
- Polysaccharide capsule
- Six different serotypes (a-f) of polysaccharide capsule
- 95% of invasive disease caused by type b (Hib)

Morphology

- Size is (1-2 X 0.3 – 0.5 microns)
- Non motile,
- Non sporing
- Gram negative rod or coccobacillus
- Pleomorphic (old culture)
- Appear as clusters of Coccobacillary forms in infected Sputum
- Long bacillary and filamentous form in infected CSF (Meningitis)

Morphology

- Stain with Carbol fuchsin (5-15min) or Loeffler's methylene blue
- Mostly Capsulated strain
- Capsule- India ink wet films and Quelling reaction

Gram staining

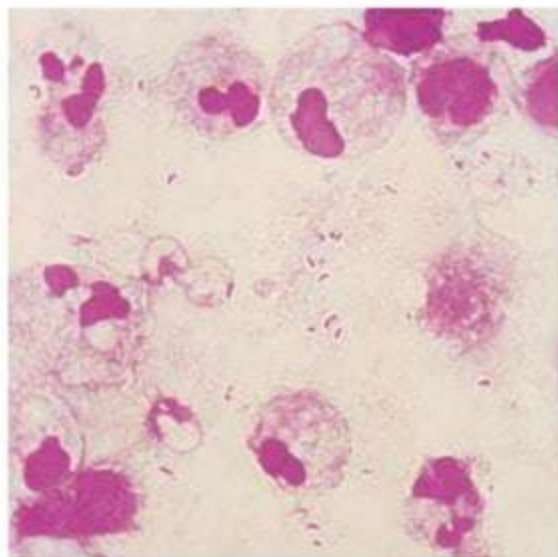
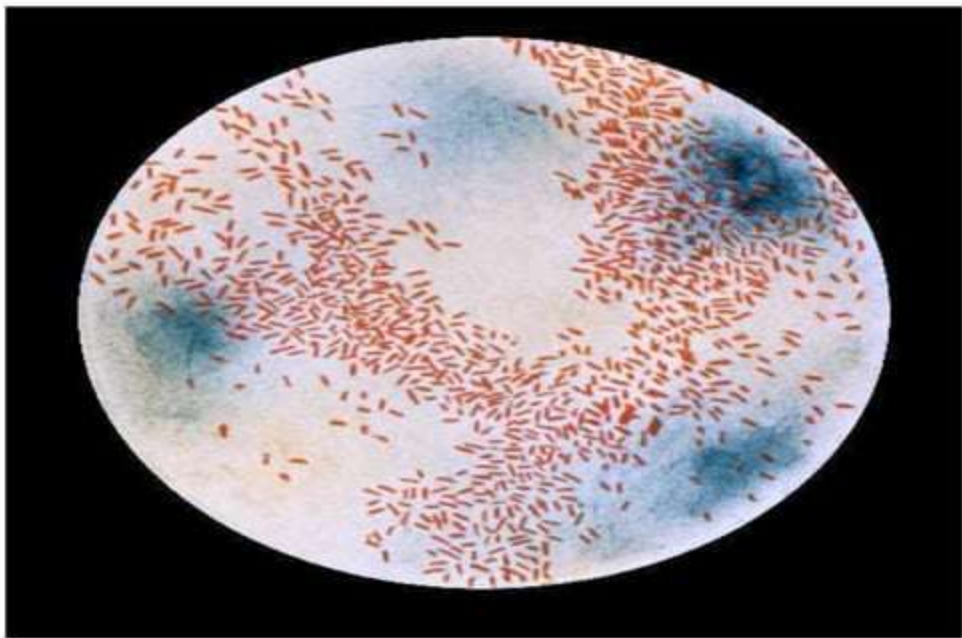


Fig: Gram-stained **CSF** sediment preparation. Fine, Gram-negative rods surrounded by a capsule (**serovar b**).
Clinical diagnosis: purulent meningitis

*Haemophilus
influenza*



Culture characteristics

- Fastidious growth requirements
- Factors X and V are essential for growth
- X is Hemin heat stable
- Porphyrins for synthesis of Cytochromes
- V-factor (NAD): Heat- labile, coenzyme I, nicotinamide adenine dinucleotide, found in blood – oxidation
- Aerobic 37 dg C

Culture characteristic

- On **Chocolate agar**, flat, grayish-brown colonies, 1-2 mm in diameter present after 24 hrs
- Colonies of staphylococci on sheep **Blood agar** cause the release of NAD, yielding satellite growth phenomenon

Satellite growth

When *Staph aureus* is streaked across plate of Blood agar with a species containing *H Influenzae*



Haemophilus Species, cont.

• Species	X	V	Hemolysis
• ► H. influenzae (H. aegyptius)	+	+	-
• ► H. parainfluenzae	-	+	-
• ► H. ducreyi	+	-	-
• ► H. haemolyticus	+	+	+
• ► H. parahaemolyticus	-	+	+
• ► H. aphrophilus	-	-	-

Biochemical reaction

- Catalase +ve
- Oxidase +ve
- Reduces nitrite to nitrate
- Ferment glucose and galactose
- Can't ferment sucrose, lactose and mannitol

Antigenic Properties

□ Contains 3 Major surface antigens

1 Capsular polysaccharide

2 Outer membrane proteins (OMP)

3 Lipopolysaccharides (LPS)

Pittman Classification

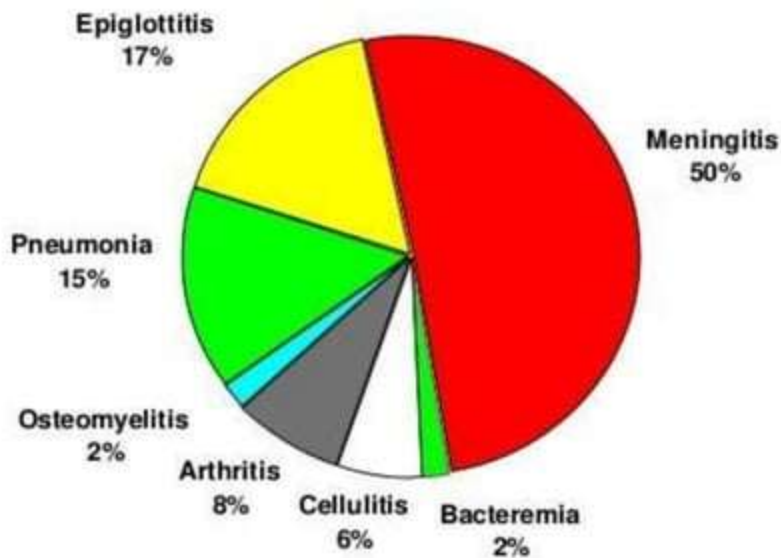
- The major antigenic determinant of capsulated strains into six capsular types **a to f**
- Typing by agglutination **Quelling** reactions precipitation and co agglutination or ELISA
- **95% of *H Influenza* isolates belong to type b**

Type b characteristics

- Has unique characters contains
- Pentose sugars
- Ribose
- Ribitol instead of Hexose in others and hexosamines
- The capsular polyribosyl ribitol phosphate (PRP) of Hib induces IgG IgM and IgA antibodies Bactericidal and opsonic and protective.
- So Hib PRP employed for Immunization

***Haemophilus influenzae* type b**

Clinical Features*



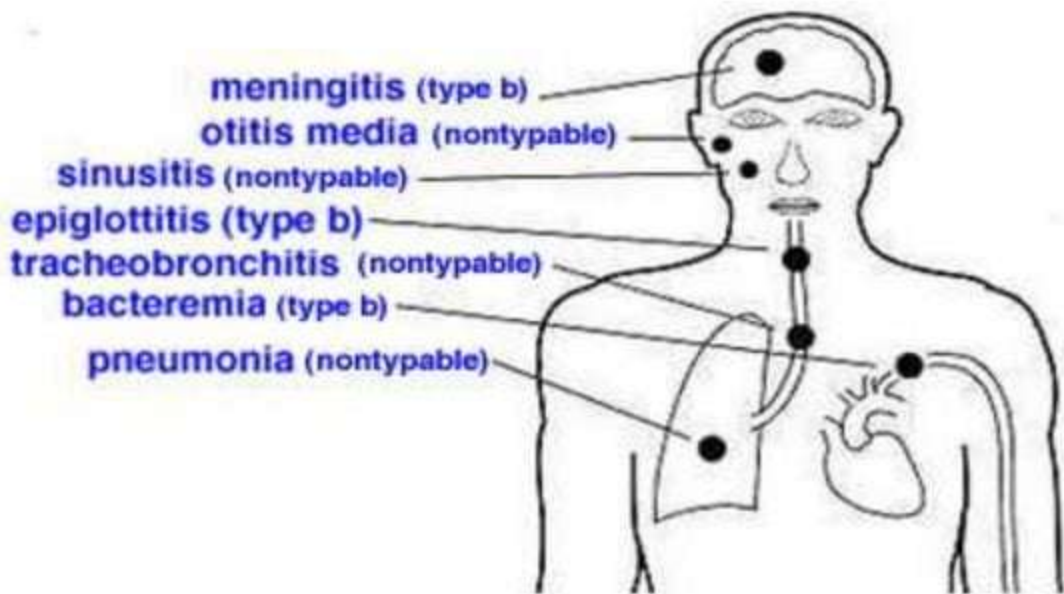
Virulence factor of *H. influenzae*

- Polysaccharid capsule
- Fimbriae
- LPS- lipid A
- All virulence strain produce **Neuraminidase** (bioflim) and **IgA protease**.
- No exotoxin

Pathogenesis

- Type b *H influenzae* colonizes the nasopharynx, and may penetrate the epithelium and capillary endothelium to cause bacteremia
- Meningitis may result from direct spread via lymphatic drainage or from hematogenous spread.
- Nontypable *H influenzae* colonizes the nasopharynx and, to a lesser extent, the trachea and bronchi and may infect mucosa damaged by viral disease.
- Lipooligosaccharide is largely responsible for inflammation

Haemophilus influenzae infections



Clinical Presentation

Pneumonia

Septic Arthritis

Epiglottitis

Meningitis

Invasion infection

Haemophilus Influenza

Mode of Transmission:

- Droplet infection and discharge from the upper respiratory tract during the infectious period.

Incubation Period

- Unknown, probably short, 2-4 days.

Infectious Period

- - As long as the organism is present, even in the absence of nasal discharge.
- - Noninfectious within 24 to 48 hours after the start of effective antibiotics

Secondary infection

- Respiratory tract infections
- Otitis media
- Sinusitis
- Chronic Bronchitis

Laboratory Diagnosis

Sample -: CSF, blood, throat swab, sputum, pus, aspirates from joints, middle ears or sinuses etc

Direct examination

- Gram staining
- Immunofluorescence and quelling reaction

Lab diagnosis

- Type b Capsular antigen detection
 - Agglutination of latex particles
 - Coagglutination test
 - Counterimmunoelectrophoresis (CIE)

Culturing and Isolation

- Can be grown on Blood agar and Chocolate agar
- Need 5 – 10 % carbon dioxide
- A streak of Staphylococcus should be streaked across the plate at 37°C
- Opaque colonies appear shows as Satellitism
- Iridescence Demonstrates on Leviathan medium
- Blood culture and CSF culture

Treatment

- Cefotaxime
- Ceftazidime
- Ampicillin, Contrimixazole
- Plasmid born resistance set in Ampicillin
- Amoxicillin with Clavulanate
- Clarithromycin
- Treatment with an effective 3rd generation cephalosporin, or chloramphenicol plus ampicillin

Ampicillin-resistant strains

Epidemiology and Prevention

- Similar to Pneumococci
- Infection enters through Respiratory tract
- Immunity is type specific
- Hib is protected by PRP vaccine
- Poorly immunogenic in children below 2 years
- Rifampicin can be given for 4 days and prevents secondary infection and eradicates carrier state.
- Haemophilus B conjugate vaccine

H. Influenzae

Meningitis (type b)

Otitis (non-typable)

Sinusitis (non-typable)

Epiglottitis (type b)

Tracheobronchitis (non-typable)

Bacteremia (type b)

Pneumonia (non-typable)

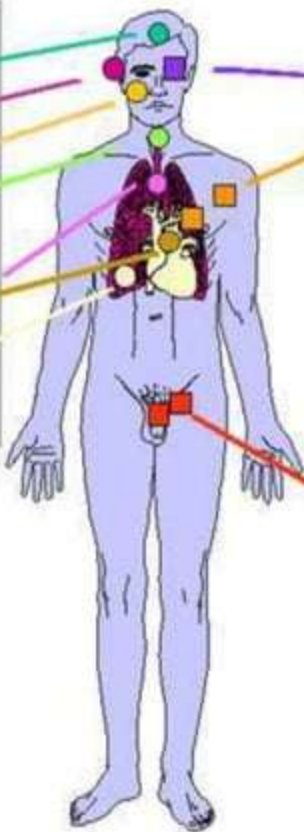
H. Aegyptius

Conjunctivitis

Purpuric fever

H. Ducreyi

Chancroid
(genitals and
lymph nodes)



Haemophilus aegyptius

- Also called Koch - Weeks Bacillus
- Gram negative coccobacillus
- Purulent conjunctivitis
- Brazilian purpuric fever
- Occur in epidemic forms
- Common in infants & children
- Respond to local sulphonamide and gentamicin

H.ducreyi

- Ducrey 1890
- Short ovoid bacilli
- 1 – 1.5 x 0.6 microns
- End to end pairing in short chains
- Gram –ve appear as Gram +ve
- Bipolar staining
- Bacilli in small groups appear as parallel chains giving school of fish appearance

H.ducreyi

- Seen in genital regions of human
- Can be transmitted by sexually contact – STD
- In men- painful ulcer in genitals, slow healing lymphnodal enlargement, pus formation- **CHANCROID**- soft based ulcer
- In women – no symptom
- Infection is localized spreading to only in regional lymph nodes

Culture of H.ducreyi

- Grows on Fresh clotted Rabbit blood
- Grows on Chorioallontoic membrane of chick embryo
- Small grey translucent colonies are produced

Chemotherapy

- Sulphonamides
- Erythromycin
- Contrimixazole
- Ciprofloxacin
- Ceftriaxone