Haemophilus influenzae

By

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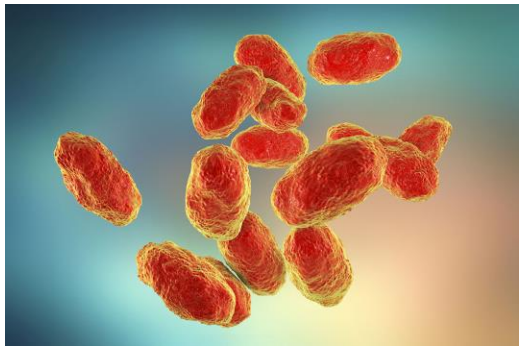
Objectives

- Identify *H. influenzae* morphology and general characters
- Know the culture and growth characters
- Understand the antigenic structure and virulence factors
- Understand the pathogenesis and disease caused by *H. influenzae*
- Diagnose diseases caused by this bacterium
- Know how to treat infection caused by this bacterium
- Understand prophylaxis measures against infections produced by this bacterium

- **Haemo-philus**

Haemo (blood)-philus (loving)

This is a group of small gram-negative coccobacilli or short rods that requires certain growth factors present in blood for their growth and *H. influenzae* is the most important human pathogen in this group.

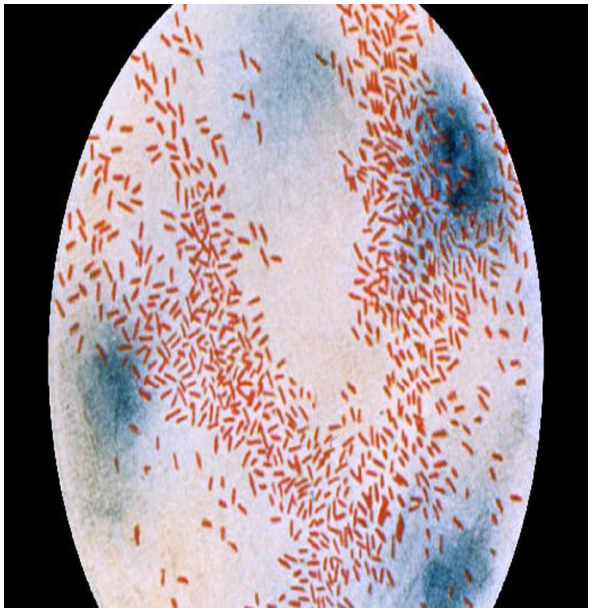


General characters

- *Haemophilus influenzae* are exclusive human bacteria found on the mucous membrane of the upper respiratory tract in humans and can live on dry hard surfaces for up to 12 days.
- Most strains of *H. influenzae* are opportunistic pathogens; they usually live in their host without causing disease, but cause problems only when other factors (such as a viral infection, reduced immune function or chronically inflamed tissues, e.g. from allergies) create an opportunity

Morphology

- Small pleomorphic gram-negative coccobacilli or short bacilli
- Generally aerobic but can grow also in anaerobic conditions (facultative anaerobe)-Non-motile, Non-spore forming.
- Virulent strains form polysaccharide capsule.



Culture and growth requirements

❑**Requires growth factors X (hemin) and V (NAD) for growth (fastidious)**

1. Factor X:

Is a heat stable factor present in blood. It is required for the synthesis of iron containing enzymes cytochrome oxidase, peroxidase and catalase.

2. V-Factor:

Is a thermolabile nicotinamide adenine dinucleotide (NAD) required in oxidation-reduction processes in the growing bacterial cell.

- These factors are present inside the erythrocytes. Heating blood till it acquires chocolate color lyses the erythrocytes thus releasing these factors.

- They grow on **chocolate blood agar (?????)** with **streaks of *Staph aureus*** which causes RBCs haemolysis and NAD production **(satellitism)**

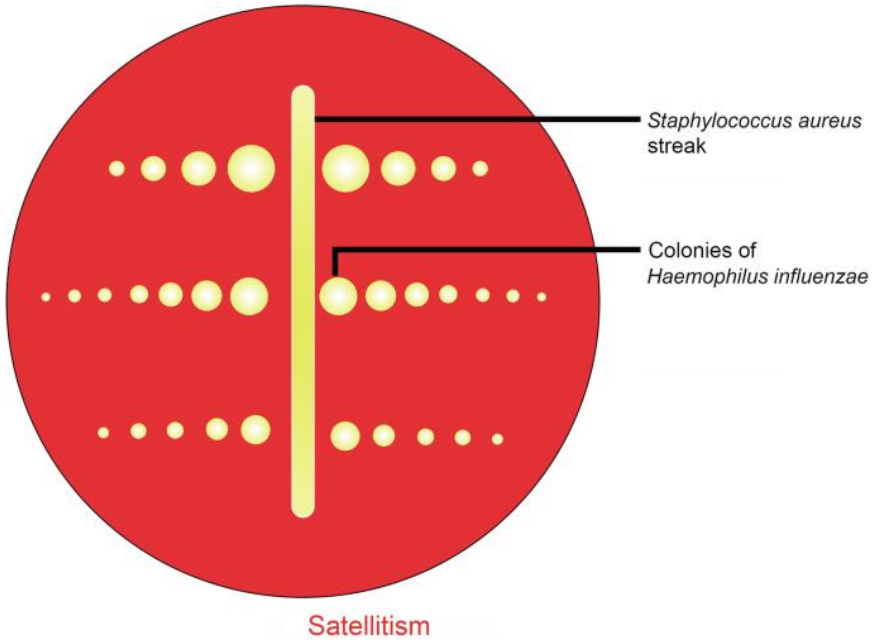
The Satellitism test

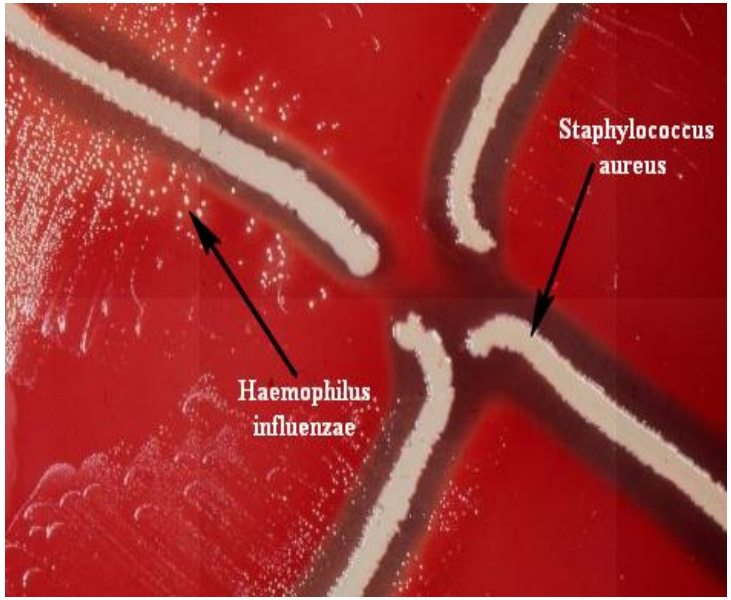
Is one of the biochemical tests used to distinguish *Haemophilus influenzae* from other *Haemophilus spp*. based on the differential requirement of the X-factor and the V-factor.

Principle of Satellitism Test

Blood agar medium provides only an X-factor, but for obtaining a V-factor, the erythrocytes present in the blood agar must be haemolyzed. H. influenzae can neither haemolyze the blood nor grow without the V-factor, so *H. influenzae* alone can't grow in a blood agar medium.

Staphylococcus aureus is hemolytic, and its presence in the blood agar medium makes V-factor (NAD) available in the medium. Hence, *H. influenzae* can grow in the vicinity of *S. aureus* colonies in the blood agar medium. This phenomenon is called 'satelliting'.





Antigenic structure and virulence factors

1. The *Haemophilus influenzae* is divided into

A. Typable (encapsulated): isolates have **capsular polysaccharides**

B. Nontypable (NTHi) (nonencapsulated): isolates lacking capsular polysaccharides and can cause noninvasive diseases.

Haemophilus that have capsule (Typable):

A. Are divided into six serotypes, designated a to f, based on the capsular polysaccharide antigen **called polyribitol phosphate (PRP)**.

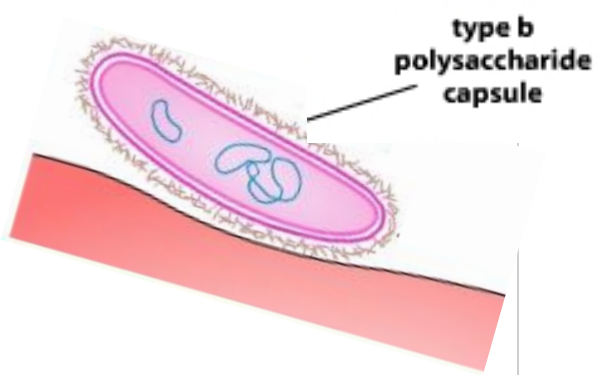
B. These capsular surface polysaccharides are strongly associated with virulence, particularly *H. influenzae* type b (Hib).

2. Lipopolysaccharide endotoxin

3. Pilli

4. IgA protease

5.Somatic outer membrane proteins



Diseases caused by *H.influenzae*

1. Meningitis

Epidemic in unvaccinated children ages 3 months to 2 years

2. Pneumonia

- 3–24 months;
Rare in vaccinated children;
- smokers

3. Bronchitis

4. Otitis media

5. Epiglottitis

Rare in vaccinated children; seen in unvaccinated toddlers

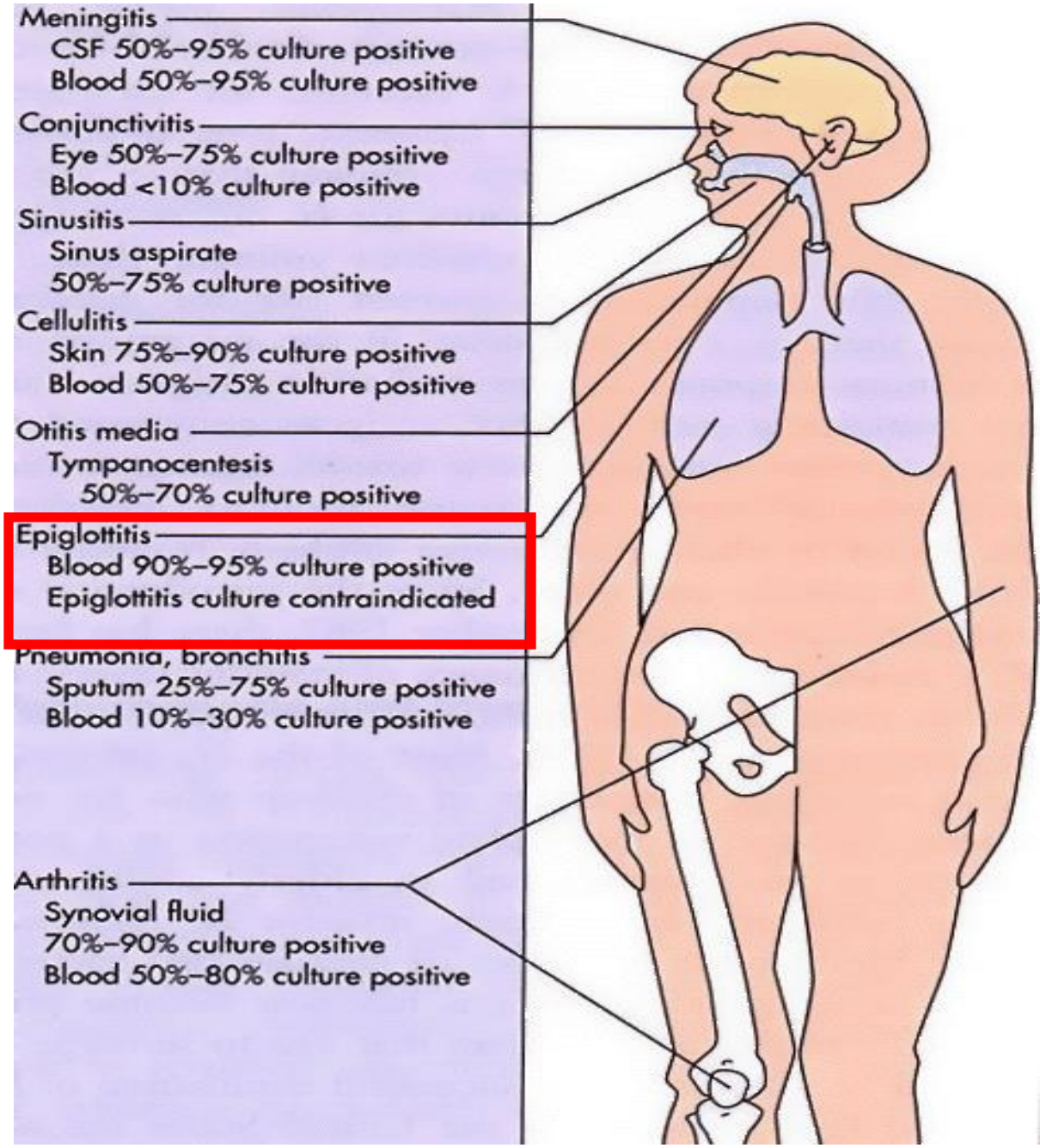
6. Cellulitis

7. Septic arthritis

Transmission—inhalation, respiratory droplets, shared tools and opportunistic.

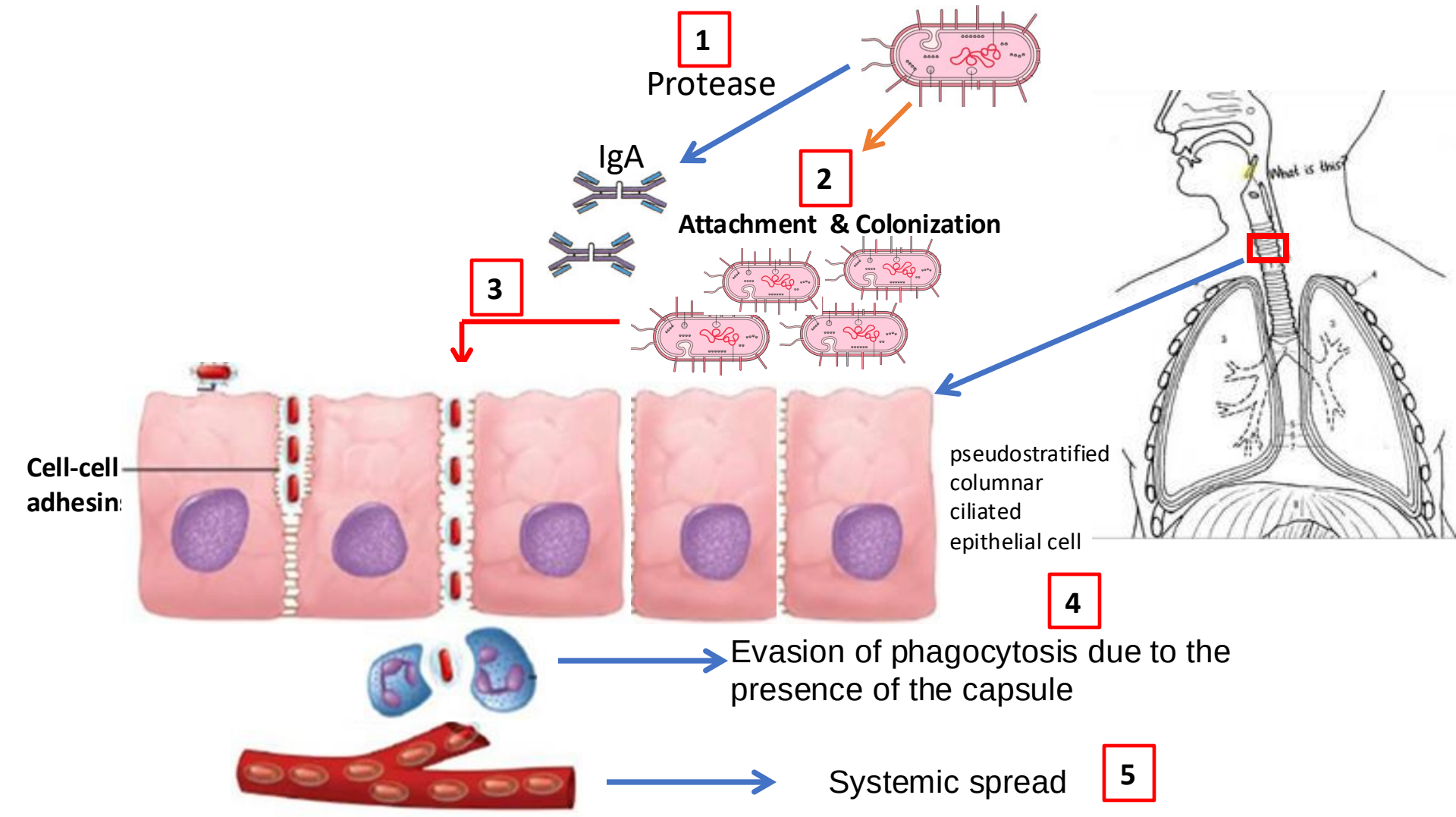
Why infection is rarely seen in the first 2 months of life ????

Diseases caused by *H. influenzae*



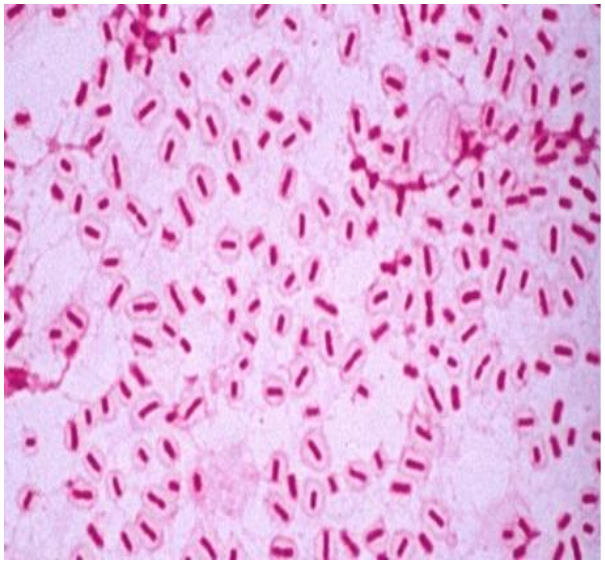
Pathogenesis of Invasive disease

The pathway of Hib reaching blood stream and causing systemic infections



Laboratory Diagnosis

- **Specimen:**- CSF, blood, sputum and pus.
- **Smear:** Gram stained, immunofluorescence and capsule swelling reaction (**Quellung reaction)**.
- **Culture:** Nutrient or Chocolate blood agar with factors x and V **(IsoVitalex enriched chocolate agar).** Addition of 10% CO_2 enhances the growth
- **Capsular polysaccharide antigen detection** by latex agglutination in CSF
- **PCR.**



Quellung reaction "swelling" and describes the microscopic appearance of *H. influenzae* capsules after their polysaccharide antigen has combined with a specific antibody. The antibody usually comes from serum taken from an immunized laboratory animal. As a result of this combination, and precipitation of the large, complex molecule formed, the capsule appears to swell, because of increased surface tension, and its outlines become demarcated.

Prophylaxis

- Hib diseases can be prevented by administration of Hib conjugate vaccine (capsular polysaccharide conjugated to carrier protein) which may be one of the following:

- **PRP-D**: the conjugated protein is *Diphtheria* toxoid.
- **HbOC:** the conjugated protein is *Corynebacterium diphtheriae* protein

-**PRP-OMP**: the conjugated protein is outer membrane protein of *Niesseria meningitidis*.

- **PRP-T:** the conjugated protein is tetanus toxoid.

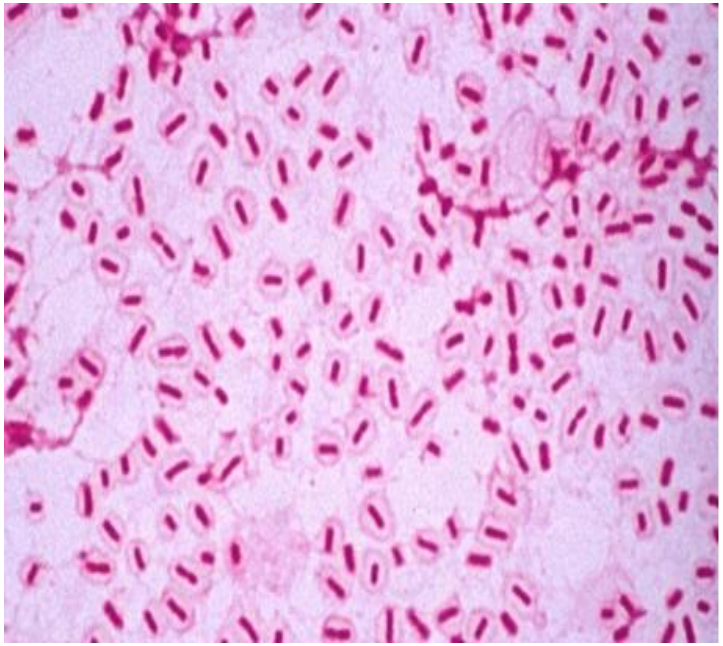
- The vaccine is given at 2,4,6 months and at 12-15 month.

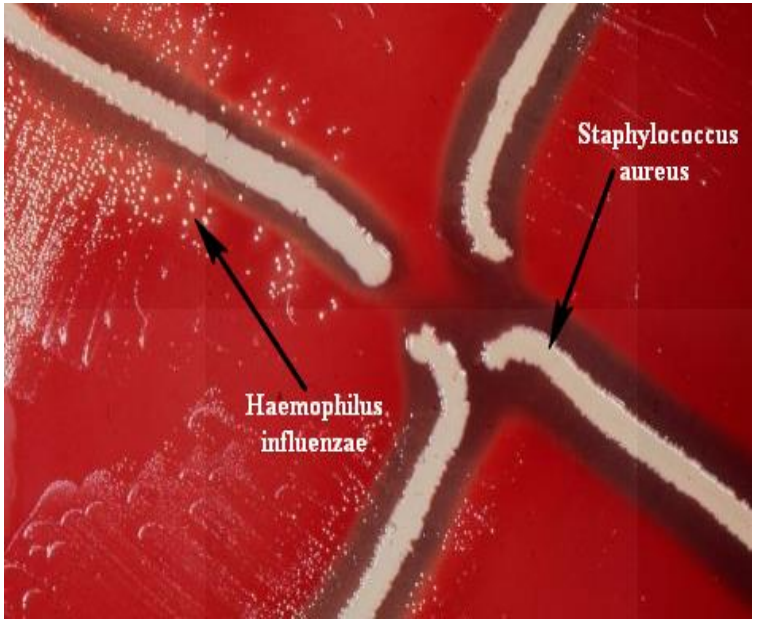
Treatment

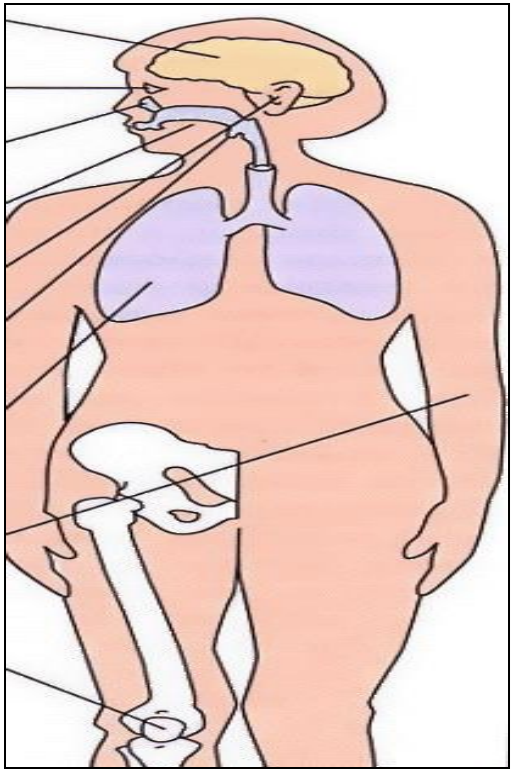
- Untreated invasive infection: Mortality rate of 90%.

Start empirically until you get sensitivity results

- Cephalosporines as cefotaxime or ceftriaxone.
- Skilled medical and nursing care is also vital in the management of acute epiglottitis, where maintenance of a patent airway is crucial.




Staphylococcus
aureus
Haemophilus
influenzae




QUIZ
TIME!

Case 1

- A 2 years old child presented to the Emergency department with two days history of being unwell with
  - **Pyrexia**
  - **Dysphagia**
  - **drooling of saliva.**
  - **Difficulty in speaking**

- What is your provisional diagnosis ?
- How to confirm your diagnosis ?
- How to treat this case ?

Case 2

- A one-year-old infant brought to the emergency room suffering from seizures, projectile vomiting, high fever after 2 days of having cough and nasal congestion.
- What is important to ask about in patient history?
- What is your provisional diagnosis?
- How to confirm the diagnosis?


Thank
You