Haemophilus influenzae
formerly called **Pfeiffer's bacillus** or *Bacillus influenzae*

It was first described in 1892 by Richard Pfeiffer during an influenza pandemic.

The bacterium was mistakenly considered to be the cause of influenza until 1933 when the viral etiology of influenza became apparent, and is still colloquially known as bacterial influenza.
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. Their capsule allows them to resist phagocytosis and complement-mediated lysis in the non-immune host.
*Unencapsulated* strains are termed nontypable (NTHi) because they lack capsular serotypes; and are almost always less invasive.
The genus Haemophilus organisms are small gram-negative coccobacilli (because rounded at ends).

Long filamentous forms also seen.

The commonest is *Haemophilus influenzae*.

The other species of the genus Haemophilus are:

1. *H. ducreyi*
2. *H. parainfluenzae*
3. *H. aegyptius*
Properties:

- It is a gram negative rod (coccobacillus).
- A facultative anaerobe which grows best in media enriched with CO₂.
- Temperature requirements 32-37 degree celcius
- Has got a polysaccharide capsule.
- Non capsulated forms also exist.
- On the basis of type of capsule there are six serotypes numbered as a, b, c, d, e and f.
- **Serotype b** is most virulent type
- Organism found only in humans.
- Virulence factors are polysaccharide capsule and endotoxin.
Lab diagnosis:

- Gram staining
- Organism is grown on chocolate agar at 37 °C in a CO₂- enriched incubator.
- Chocolate agar is enriched with two factors.
  1. Factor X (haematin)
  2. Factor V (NAD).
- Other species do not require both factors
  The colonies will be greyish-white, small, and mucoid.
- Catalase and oxidase Positive
Definitive diagnosis can be made with **Quellung test**.

Additional means of identifying encapsulated strains include **fluorescent-antibody staining** of the organism and **latex agglutination tests**, which detect the capsular polysaccharide. **Polymerase chain reaction (PCR)** assays have been proven to be more sensitive than either Quellung test or culture tests, and highly specific.
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.

However, *H. influenzae* isolated from **cerebrospinal fluid** or **blood** would indicate *H. influenzae* infection.
Bordetella
Bordetella is placed among the Gram-negative aerobic rods and cocci.

The genus Bordetella contains seven species. **B. pertussis** is by far the most important causative agent of whooping cough.

Other important ones are **B. parapertussis**, **B. bronchoseptica**.

The bacteria are nutritionally **fastidious** and are usually cultivated on rich media supplemented with **blood**. Even on blood agar the organism grows slowly and requires **3-6 days** to form **pinpoint** colonies.
Properties:

- *B. pertussis* is a tiny (0.5 to 1.0 m), gram-negative cocco-bacillary rod. With toludine blue stain, bipolar metachromactic granules can be demonstrated.

- **Encapsulated**

- **Obligate aerobe**

- The organism is also very susceptible to environmental changes and survives for little time outside the human respiratory tract.

- **Oxidase** and **Catalase** positive.

- The pilli of cell wall contain a protein called filamentous haemagglutinin (fha)
Lab diagnosis:

- Gram negative staining.
- Obligate aerobic; highly fastidious, or difficult to culture.
- The organism can be isolated from nasopharyngeal swabs taken during the paroxysmal stage. **Bordet-Gengou medium** or **Regan-Lowe** is used for the culture.
- Direct fluorescent-antibody staining of the nasopharyngeal specimens is often used for diagnosis.
- Polymerase Chain Reaction
BRUCELLA
Brucella

- Are obligate parasites of animals and humans
- Are characteristically located intracellularly
- Species:
  1. *Brucella melitensis* ➔ infects goats
  2. *Brucella suis* ➔ infects swine
  3. *Brucella abortus* ➔ infects cattle
  4. *Brucella canis* ➔ infects dogs

The disease in humans called **Brucellosis** (Undulant fever, Malta fever) is characterized by an acute bacteremiac phase followed by chronic stage that may extend over many years and may involve many tissues
Specimens

- Blood should be taken for culture
- Biopsy material for culture
- Serum for serologic tests
Culture

- Brucella agar was specifically designed to culture Brucella species bacteria.
- Brucella species bacteria grow on trypticase-soy medium with or without 5% sheep blood, brain-heart infusion medium.
- Brucella species bacteria grow on chocolate agar and blood agar.
- All culture should be incubated in 8 – 10% CO$_2$ at 35 – 37°C and should be observed for 3 weeks.

Small, convex, smooth colonies appear on enriched media in 2 – 5 days.
Christensen’s urea slant should be inoculated. A positive urease test result is characteristic of *Brucella* species

- *Brucella suis* and some strains of *Brucella melitensis* can yield a positive test result less than 5 minutes after inoculating the slant. Other strains take a few hours to 24 hours.

- **The principle of the test:**

  The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive from other *Enterobacteriaceae*. 
- **Urease test media** contain 2% **urea** and **phenol red** as a pH indicator. An increase in pH due to the production of ammonia results in a color change from yellow (pH 6.8) to bright pink (pH 8.2).

- **Urea broth** (Stuart’s urea broth) is a highly buffered medium requiring large quantities of ammonia to raise the pH above 8.0 resulting in a color change. This medium provides all essential nutrients for **Proteus**, for which it is differential.

- **Urea agar** (Christensen’s urea agar) has a reduced buffer content and contains peptones and glucose. This medium supports the growth of many enterobacteria allowing for the observation of urease activity.
**Serology**

- Immunoglobulin IgM antibody levels rise during the first week of acute illness, peak at 3 months, and may persist during chronic disease.

- Immunoglobulin IgG antibody levels rise about 3 weeks after onset of acute disease, peak at 6 – 8 weeks, and remain high during chronic disease.
- Agglutination test
- IgG agglutinin titers above 1:80 indicate active infection
- ELISA assays
Yersinia
- Non lactose fermenting Gram negative rods
- Motile (except *Y. pestis*)
- 10 species, only *Y. pestis* (plague), *Y. enterocolitica*, *Y. pseudotuberculosis* (enteric disease, septicaemia) pathogenic for animals and man
- Demonstrate bipolar staining in Giemsa stained smears from animal tissues
- Serotyping and biotyping used to discriminate between strains
- 10 serotypes of *Y. pseudotuberculosis*, serotypes I, II and III contain majority of pathogenic isolates
- 5 biotypes and more than 50 serotypes of *Y. enterocolitica*
**Diagnosis**

- Histological examination of intestinal lesions
- Giemsa stained smears of pus reveal large numbers of bipolar rods
- Culture of *Y. enterocolitica* and *Y. pseudotuberculosis* from faeces, pus or tissue
- Plated on MacConkey agar for growth at 37 or room temperature
- DFA tests (direct fluorescent antibody)
- API 20E
- Serotyping
- **Yersinia species**
- **Y. pseudotuberculosis** and **Y. enterocolitica** in intestinal tract of wild mammals, birds and domestic animals
- All may be reservoirs of infection
- Many avian species may act as amplifier hosts and may transfer the organisms mechanically
- Both organisms grow in a wide temperature range (5-42 degrees)
- In endemic areas wild rodents are important reservoirs of **Y. pestis**. Fleas, especially the Oriental rat flea transmit the infection to man and other animals