Assist. Prof. Dr. Ameer Mezher Hadi
Dr.Zaid Abdel Hadi Abd
Lecture. 10
Second Stage

College of Health & Medical Techniques
Department of Medical Laboratories Techniques

HAEMOPHILUS

General Characteristics

The genus *Haemophilus* contains significant genetic diversity. **Members of the genus are small, nonmotile, pleomorphic gram-negative bacilli.** The cells are typically coccobacillary or short rods. Species of the genus *Haemophilus* **require protoporphyrin IX (a metabolic intermediate of the hemin biosynthetic pathway)**, referred to as **X factor** and **V factor**, **nicotine adenine dinucleotide (NAD),** or nicotine adenine dinucleotide phosphate (NADP) for *in vitro* growth. *Haemophilus* spp. are facultative anaerobes enhanced in a 5% to 7% CO 2-enriched atmosphere. The morphologic and physiologic features of individual species are presented in the discussion of laboratory diagnosis.

Epidemiology

As presented in Table 1, except for *Haemophilus ducreyi*, *Haemophilus* spp. normally inhabit the upper respiratory tract of humans. Asymptomatic colonization with *Haemophilus influenzae* type b is rare. Although *H. ducreyi* is only found in humans, the organism is not part of our normal microbiota, and its presence in clinical specimens indicates infection.

Table-1: general characteristics of *Haemophilus species*

Organism	Habitat (Reservoir)	Mode of Transmission
Haemophilus influenzae	Normal microbiota: upper respiratory tract	Person-toperson: respiratory droplets Endogenous strains

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Haemophilus ducreyi	Not part of normal human microbiota; only found in humans during infection	Person-toperson: sexual contact
Other *Haemophilus* spp.*Haemophilus* parainfluenzae*Haemophilus* parahaemolyticus	Normal microbiota: upper respiratory tract	Endogenous strains

Pathogenesis and Spectrum of Disease

Production of a capsule and factors that mediate bacterial attachment to human epithelial cells are the primary virulence factors associated with *Haemophilus* spp. In general, infections caused by ***H. influenzae*** **are often systemic and life-threatening**, whereas infections caused by nontypeable (do not have a capsule) strains are usually localized. Most serious infections caused by ***H. influenzae*** **type b are biotypes I and II.**

Most *H. influenzae* infections are now **caused by nontypeable strains (NTHi).** Transmission is often via respiratory secretions. The organism is able to gain access to sterile sites from colonization in the upper respiratory tract. **Clinical infections include otitis media (ear infection), sinusitis, bronchitis, pneumonia, and conjunctivitis**. Immunodeficiencies and chronic respiratory problems such as chronic obstructive pulmonary disease may predispose an individual to infection with NTHi.

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Chancroid is the sexually transmitted disease caused by *H. ducreyi* . The initial symptom is the development of a painful genital ulcer and inguinal lymphadenopathy.

Table-2: virulence factor of *Haemophilus* and the disease that caused

Organism	Virulence Factors	Spectrum of Disease and Infections
Haemophilus influenzae	Capsule: Antiphagocytic, type b most common. Additional cell envelope factors mediate attachment to host cells. Unencapsulated strains: pili and other cell surface factors mediate attachment.	Encapsulated strains: Meningitis Epiglottitis Cellulitis with bacteremia Septic arthritis Pneumonia Nonencapsulated strains: Localized infections Otitis media Sinusitis Conjunctivitis Immunocompromised patients: Chronic bronchitis Pneumonia Bacteremia
Haemophilus influenzae	Uncertain; probably similar to those of other *H. influenzae.*	Purulent conjunctivitis single strain identified as the Brazilian purpuric fever, high mortality in children between ages 1 and 4; infection includes purulent meningitis, bacteremia, high fever, vomiting, purpura (i.e., rash), and vascular collapse.
Haemophilus ducreyi	Uncertain, but capsular factors, pili, and certain toxins are probably involved in attachment and penetration of host epithelial cells.	Chancroid; genital lesions progress from tender papules (i.e., small bumps) to painful ulcers with several satellite lesions; regional lymphadenitis is common.

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Laboratory Diagnosis

Specimens

Specimens consist of expectorated sputum and other types of **respiratory specimens, pus, blood, and spinal** fluid for smears and cultures depending on the source of the infection.

Direct Observation

To increase the sensitivity of direct Gram stain examination of body fluid specimens, especially CSF, specimens may be centrifuged (2000 rpm for 10 minutes), and the smear is prepared from the pellet deposited in the bottom of the tube. Gram stains of the smears from clinical specimens must be examined carefully. *Haemophilus* spp. stain a pale pink and may be difficult to detect in the pink background of proteinaceous material often found in clinical specimens.

Antigen Detection

H. influenzae type b capsular polysaccharide in clinical specimens, such as CSF and urine, can be detected directly using commercially available particle agglutination assays.

Molecular Methods

Rapid screening procedures are very useful for patient therapy and evaluating outbreaks and have been developed for detection from CSF, plasma, serum, and whole blood. A polymerase chain reaction (PCR) for *H. influenzae* capsular types a and f has been developed.

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Incubation Conditions and Duration

Most strains of *Haemophilus* spp. are able to grow aerobically and anaerobically (facultative anaerobes). **Growth is stimulated by 5% to 10% carbon dioxide (CO2).** It is recommended that **cultures be incubated in a candle jar, CO2 pouch, or CO2 incubator.**

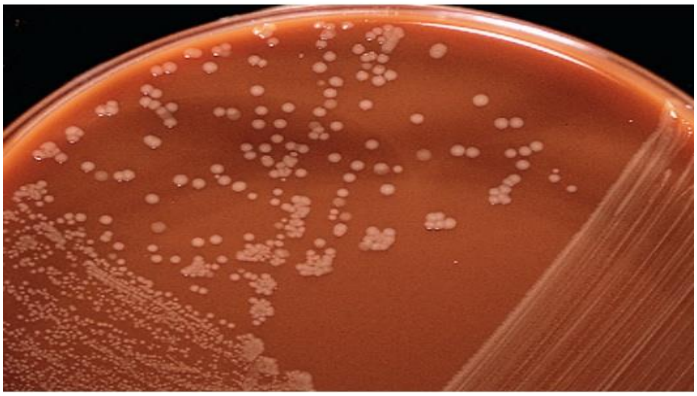

Figure-1: *Example of Haemophilus* influenzae growing on chocolate (CHOC) agar. Notice the tan mucoid colonies characteristic of encapsulated strains

Cultivation / Media of Choice

Haemophilus spp. typically grow on chocolate agar as smooth, flat or convex, buff or slightly yellow colonies. **Chocolate agar provides hemin (X factor) and NAD (V factor),** necessary for the growth of *Haemophilus* spp. Most strains will not grow on 5% sheep blood agar, which contains protoporphyrin IX but not NAD. Several bacterial species, including Staphylococcus aureus, produce NAD as a metabolic byproduct. Therefore, tiny colonies of *Haemophilus* spp. may be seen growing on sheep blood agar

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very close to colonies of bacteria capable of producing V factor; this is known as the **satellite phenomenon.**

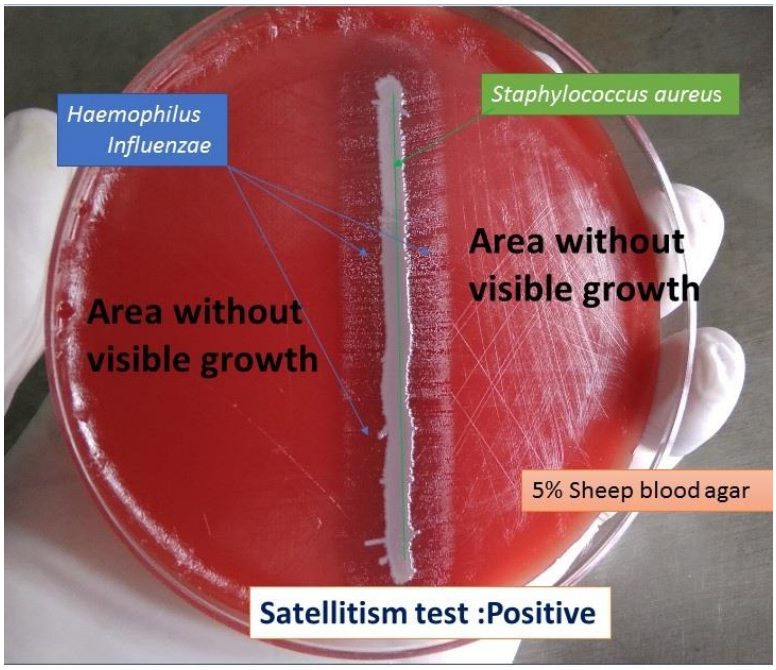

Figure-2: ***Haemophilus influenzae satellite phenomenon***

Treatment

Invasive *H. influenzae* infection often requires hospitalization. The current recommended treatment of life-threatening illness caused by *H. influenzae* is cefotaxime or ceftriaxone. Alternative drugs include trimethoprim-sulfamethoxazole, imipenem, and ciprofloxacin.