

Coccobacilli

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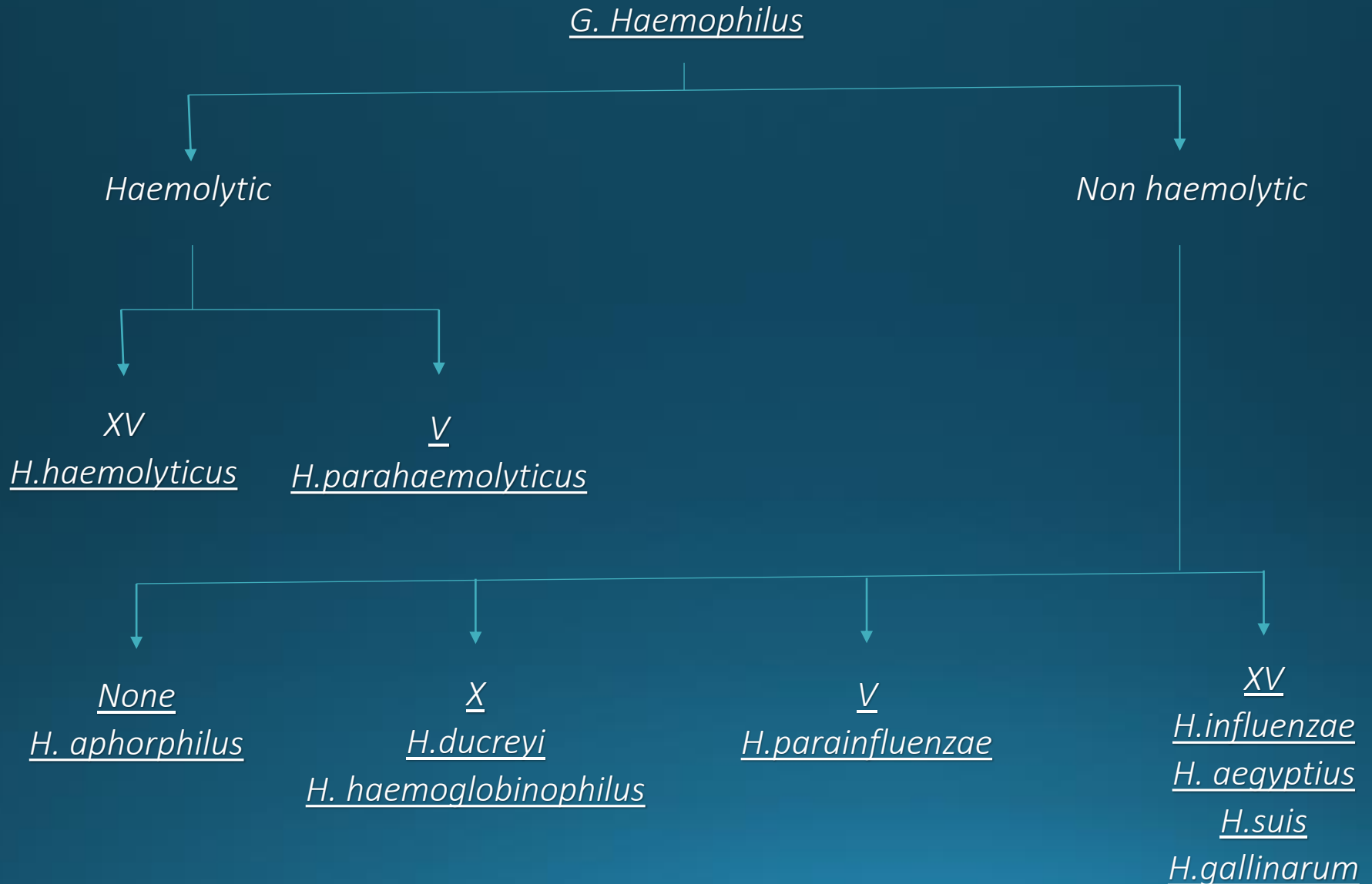
Intermediate shape between cocci (spherical bacteria) and bacilli (rod-shaped bacteria)

The important genera that belong to different

Families are:

- 1. G. Haemophilus*
- 2. G. Bordetella*
- 3. G. Brucella*
- 4. G. Yersinia*

Genus: Haemophilus classification of G. haemophilus



Haemophilus influenzae

- **Morphology**
- *Haemophilus influenzae* (formerly called Pfeiffer's bacillus or *Bacillus influenzae*) is a Gram-negative, coccobacillary, facultatively anaerobic bacterium belonging to the *Pasteurellaceae* family.

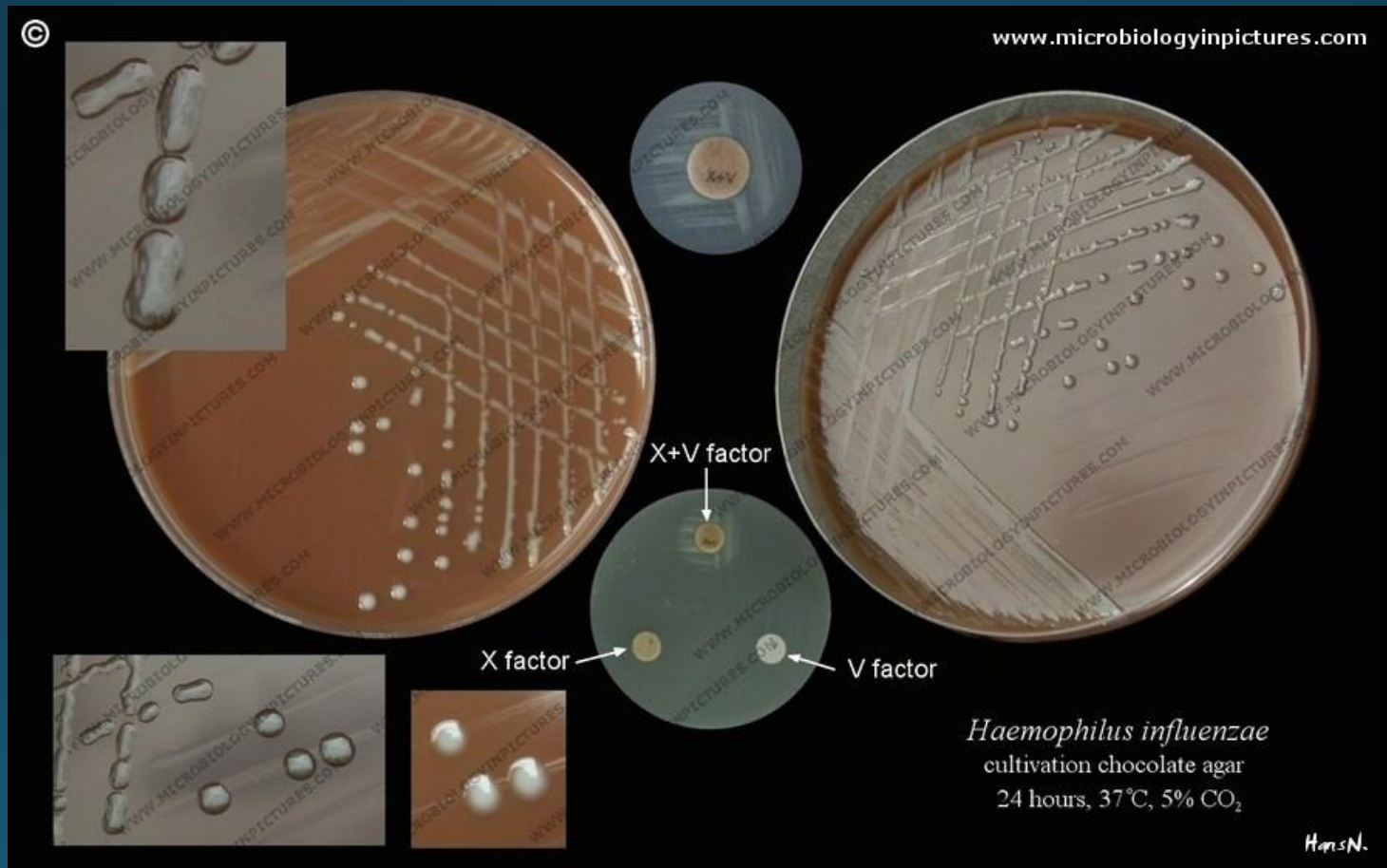


Culture Characteristics and diagnosis

- Bacterial culture of *H. influenzae* is performed on agar plates, the preferable one being chocolate agar, with added X (hemin) and V (nicotinamide adenine dinucleotide) factors at 37°C in a CO₂-enriched incubator. Colonies of *H. influenzae* appear as convex, smooth, pale, grey or transparent colonies.

Culture Characteristics and diagnosis

chocolate agar



Culture Characteristics and diagnosis

- The cultured organism can be further characterized using catalase and oxidase tests, both of which should be positive. Further serological testing is necessary to distinguish the capsular polysaccharide and differentiate between *H. influenzae* b and nonencapsulated species.



Culture Characteristics and diagnosis

- Blood agar growth is only achieved as a satellite phenomenon around other bacteria.
- *H. influenzae* will grow in the hemolytic zone of *Staphylococcus aureus* on blood agar plates; the hemolysis of cells by *S. aureus* releases factor V which is needed for its growth.
- *H. influenzae* will not grow outside the hemolytic zone of *S. aureus* due to the lack of nutrients such as factor V in these areas.

Culture Characteristics and diagnosis

Blood agar



Culture Characteristics and diagnosis

Blood agar and chocolate agar



Ahmed Abdul Redha Alkazaz

Genus: *Brucella*

- The name “*Brucella*” was derived from the name of the scientist “Sir David Bruce” who discovered it to be causative agent of undulant fever. This disease is also called “Malta Fever” because it was prevalent in Island of Malta. Undulant fever can be also called “Brucellosis”.
- Brucellosis, which is a zoonosis. It is transmitted by ingesting contaminated food (such as unpasteurized milk products), direct contact with an infected animal, or inhalation of aerosols.
- The important Species of genus *Brucella*:
 - 1. *B. abortus* : cattle
 - 2. *B. melitensis*: sheep and goat
 - 3. *B. suis*: pig

Morphology

- Genetically genus *Brucella* is of a single species which is *B. melitensis* and all other m.o. are biovars of this species.
- The typical m.o. are predominantly gram negative coccobacilli, and are capsulated, which function as facultative intracellular parasites.



Culture Characteristics

- Strict aerobes

Fastidious and nutritional requirement are complex

Grow best in medium enriched with glucose and animal serum

The optimum temperature is 37°C and pH 6.6 to 7.4

Addition of 5-10% CO₂ improves the growth of *B. abortus* and *B. melitensis*

Addition of bacitracin, polymyxin or cycloheximide makes media selective for brucellae



Biochemical reactions

- Do not ferment carbohydrate
Catalase positive, oxidase positive and urease positive
Reduce nitrate to nitrites
Citrate, VP negative and do not produce indole

Laboratory Diagnosis

- **Specimen:**
- Blood
- Urine, sputum, breast milk
- Lymph node biopsy
- Bone marrow aspirate
- **Microscopy is insensitive**
- **Culture and isolation:**
- *Liver infusion or glucose serum broth or trypticase soya broth:* At least 10 cc blood or biopsied material is cultured in a bottle of liver infusion or glucose serum broth or trypticase soya broth and incubated at 37 c under 5-10% CO₂. Subcultures are made on liver infusion agar or trypticase soya agar every 3-5 days.
- ***Castaneda's method:*** Castaneda's media provides both liquid (liver infusion broth) and solid media (3% nutrient agar slope) in one bottle. Since the bottle contains both liquid and solid media, the broth flows over the surface of agar slant when bottle is tilted resulting in automatic subculture.
- ***Bactec:*** The BACTEC MYCO/F LYTIC (MFL) medium has been recently developed to improve the recovery of intracellular pathogens such as fungi and mycobacteria.

Identification

- Requirement of CO₂ for growth
- Production of urease and H₂S
- Sensitivity to the aniline dyes: fuschin, thionine (B. melitensis is not inhibited by any one of the dyes, whereas B. abortus is inhibited by thionine and not by fuschin and B. suis is inhibited by fuschin but not by thionine)
- Agglutination with monospecific sera

Serology

- *Standard agglutination test (SAT)*: Diluted serum of patients is tested by slide or tube method against a brucella suspension. Both IgM and IgG continue to rise during acute stage of infection. A presumptive diagnosis can be made when there is 4 fold rise in the titre or a single titre greater than 80.
Prozone phenomenon: absence of agglutination in low dilution of serum due to high concentration of antibodies
- *ELISA and RIA*: can distinguish IgM, IgG and IgA brucella specific antibodies
- **Brucellin test (Skin test)**: Positive reaction shows an area of erythema and induration of 6 mm diameter within 24 hours.

Latex particle agglutination

- The latex particle agglutination test (LAT) is a more sensitive method used in diagnosis. Because the method relies on antigen rather than viable bacteria, the results are not disrupted by prior antibiotic use. It also has the added benefit of being much quicker than other methods. However, antibiotic sensitivity testing is not possible with LAT alone, so a parallel culture is necessary.



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Genus: *Bordetella*

- Morphology
- *Bordetella pertussis* is a Gram-negative, aerobic coccobacillus capsule of the genus *Bordetella*, Unlike *B. bronchiseptica*, *B. pertussis* is nonmotile. diplococci arrangement



Culture Characteristics

- Because it is a fastidious m.o., it requires enriched media for its cultivation which are:
- 1. For primary isolation; an enrichment media is used. This medium is called Bordet-Gengou (potato-blood-glycerol agar). The medium contains 20-30 % sheep blood cells and penicillin -G or methicillin to make more selective.



Culture Characteristics

- 2. Buffered charcoal yeast extract agar (BCYE); this medium can also be used for the isolation of *Legionella pneumophila*



Culture Characteristics

- 3. Blood Charcoal Agar (BCA) to which an antibiotic may be added as cephalixin to make more selective (inhibits respiratory flora).
- 4. Regan-Lowe agar which is similar to BCA, but contains a half strength of charcoal.
- 5. Charcoal and cephalixin.

Cultural requirement & cultural characteristics

- This m.o. is a strict aerobe, best grow at 35-37 C for 3-7 days, and the media should be kept in moist environment (in sealed plastic bag).
- **Growth (cultural) characteristics:**
- The colonies appear as rounded, convex,
- 1-2 mm in diameter, and look like mercury drops or pearl-like appearance. The colonies have iridescence and surrounded by narrow zones of haemolysis.
- Biochemically; the m.o. can ferment glucose with acid, but no gas production. Also, these m.o. do not require neither X nor V factors.

Diagnosis

1. A nasopharyngeal or an oropharynx swab is sent to the bacteriology laboratory for Gram stain.
2. growth on Bordet-Gengou agar or BCYE plate with added cephalosporin to select for the organism.
3. Several diagnostic tests are available, especially ELISA kits.

Тяжело!