MICROBIOLOGY LAB . 8

Gram- positive rods

Non-spore formers

Corynebacterium & Listeria

lect.
Shaima'a Al-Salihy

Learning Objectives:

After this lab. You must be able to:

- Distinguish between *Corynebacterium* and *Listeria*
- Describe the two genera microscopically and culturally.
- List types of clinical infections these organisms produce
- Predict G +ve causative agents causing clinical cases.
- Discuss the principles of identifying tests.
- Know the prevention ways of each organism.

Non spore-forming Gram-positive bacilli

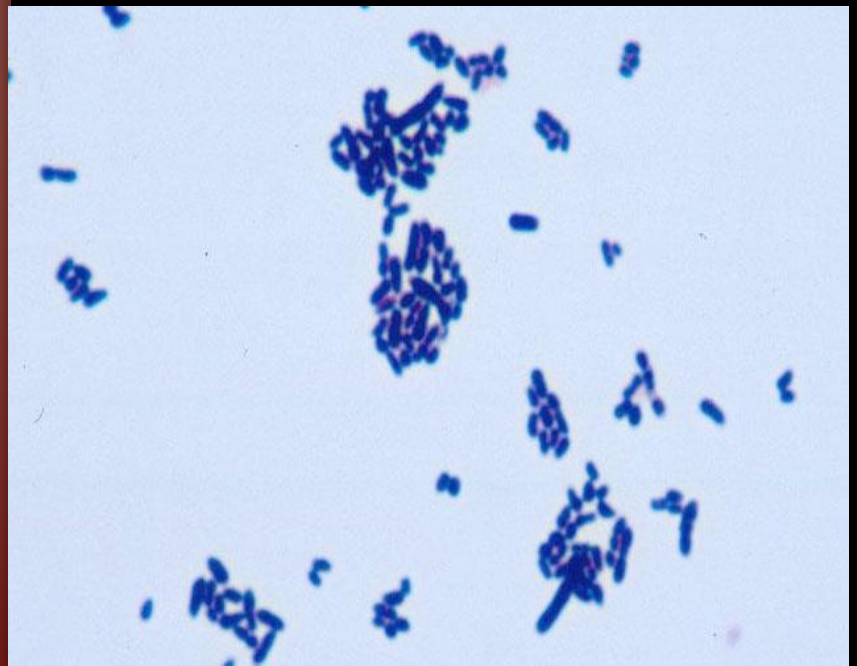
There are two important pathogens in this group:

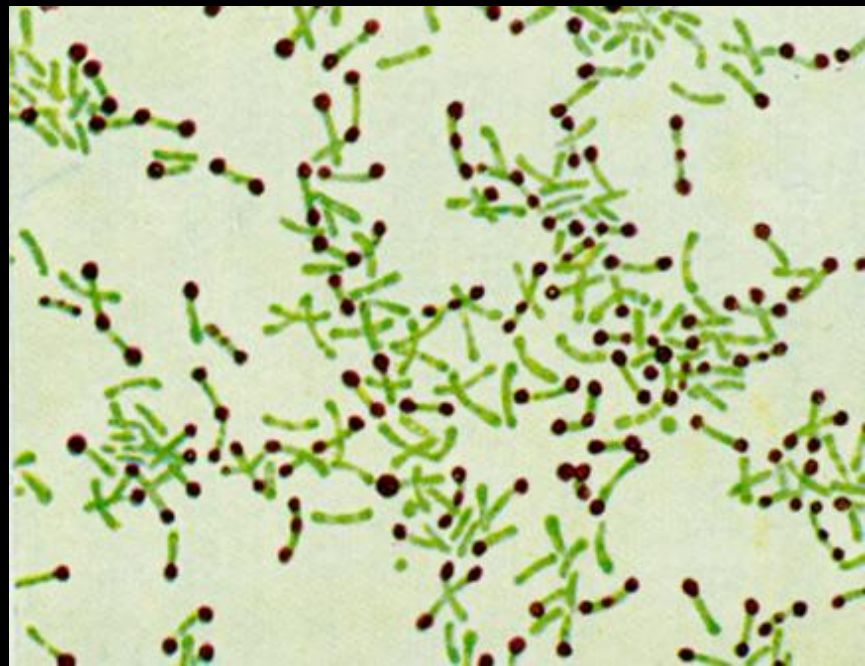
- ***Corynebacterium diphtheriae***
- ***Listeria monocytogenes***

Corynebacterium diphtheriae

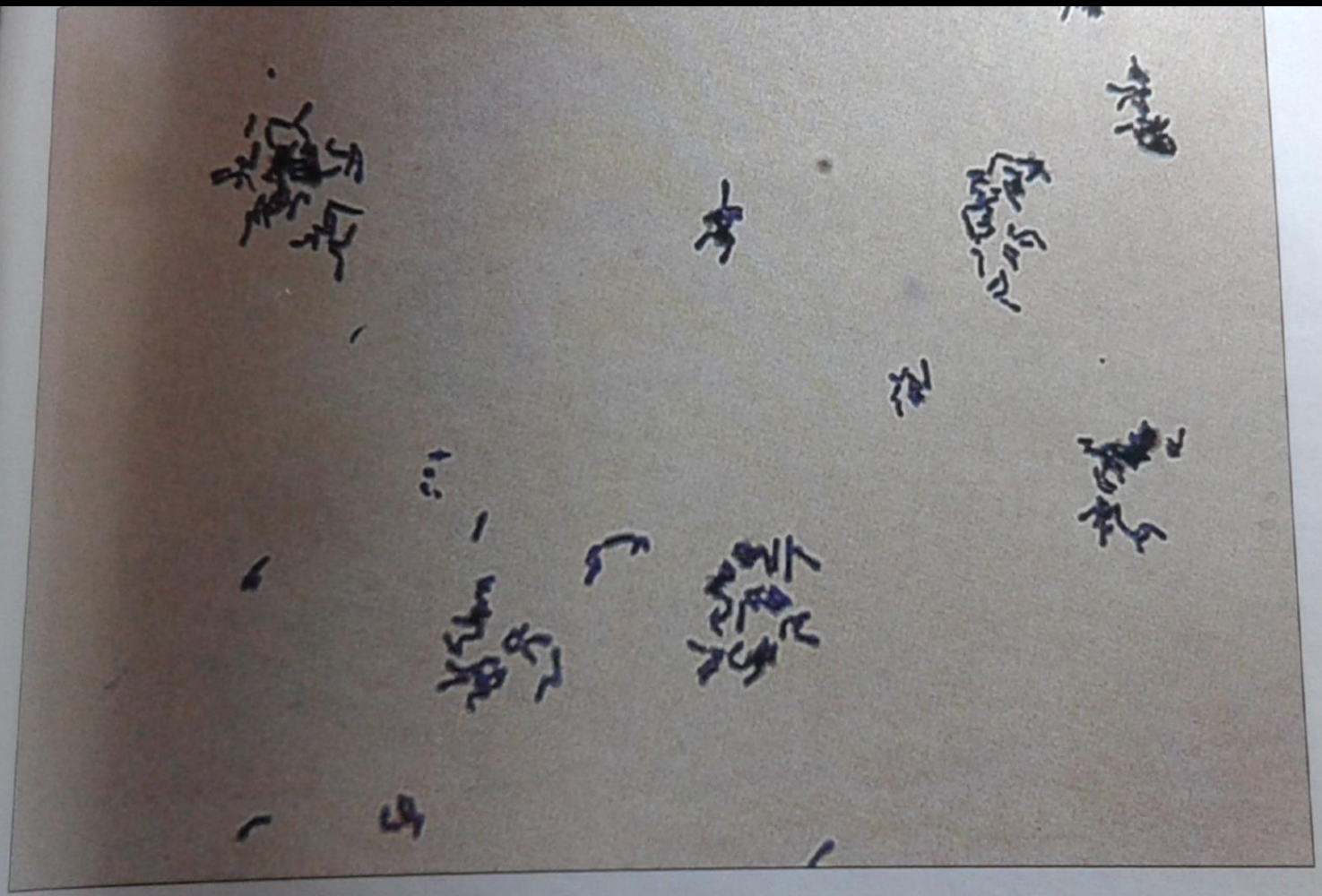
Important properties:

- G +ve rods, that appear club-shaped,
- Arranged in cueniform or Chinese letters and are at obtuse angles to each other (V or L shaped).
- Have metachromatic granules*.
- Non motile, non sporing, and non capsulated.
- Pleomorphic.





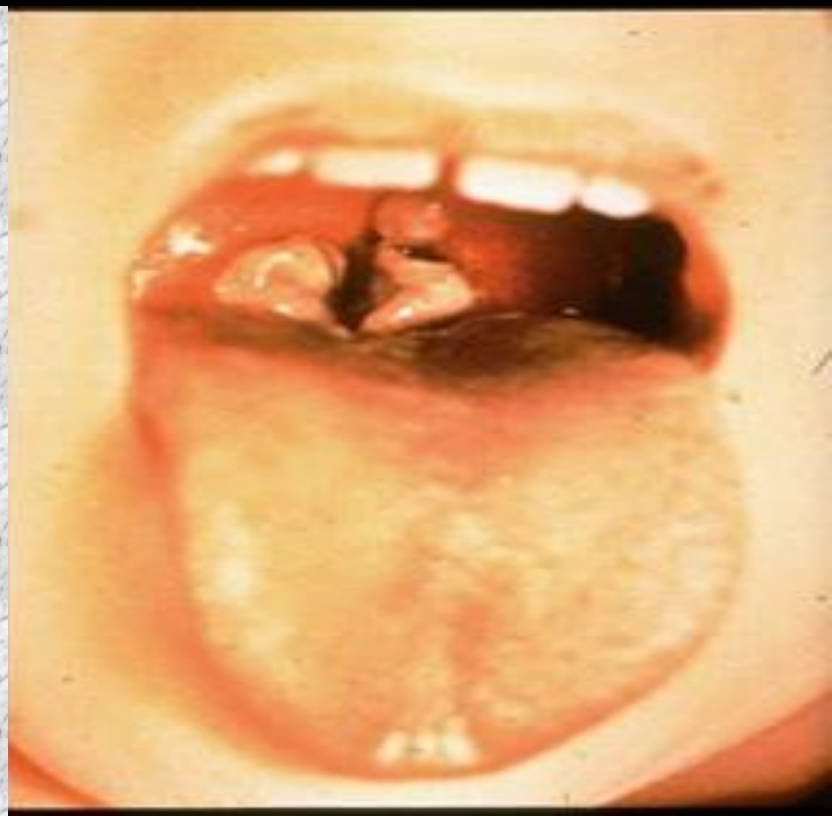
Gram stain of diphtheroids



142 **Gram stain of diphtheroids.** The term 'diphtheroids' includes various *Corynebacterium* species that are skin commensals. The Gram stain shows a typical 'Chinese lettering' arrangement of the bacilli. *(Gram stain, ×1000)*

Clinical findings:

- ✓ *Respiratory diphtheria: Thick, gray, adherent **pseudomembrane** over the tonsils and throat.
- ✓ Cutaneous diphtheria: ulcerating skin lesion covered by a gray membrane.



Laboratory diagnosis:

- Specimens: throat swab or swab from pseudomembrane
- Microscopy:

- Staining by Gram or methylene blue (G +ve rods arranged as L or V shaped)

- Albert stain: differential stain for metachromatic granules.

- Culture:

- Loeffler's serum slope:* creamy white colonies in 6-8 hrs.

Advantages of Loeffler's medium:

1- growth is very fast.

2- metachromatic granules are seen better.

3- collect fluid at the base of the medium which cauld be used in toxigenicity and biochemical test

4- checking proper collection of throat swabs.

-Potassium tellurite medium (McLeod's medium): black colonies. Because this agar contain tellurite that is reduced to elemental tellurium within the organism.

Advantages of McLeod's medium:

1- It is a selective medium

2- It gives a discrete colonies of *C. diphtheriae.*

3- It can differentiate strains of *C. diphtheriae**

4- It can be used to isolate *C. diphtheriae* from carriers even the small number of bacteria are present.

- Tinsdale's cystine-sodium thiosulphate tellurite serum agar: grey black colonies surrounded by a dark brown halo due to the formation of H2S from cystine by the action of cystinase present in *C. diphtheriae*

Toxigenicity test:

1- in vivo: animal inoculation, Schick test.

2- in vitro: (Elek's test) antibody-based gel diffusion precipitation test to confirm toxin production.

3- tissue culture neutralization assay.

4- PCR assay: for the presence of toxin gene in the organism isolated from the patient.

143 ***Corynebacterium diphtheriae*, tellurite blood medium.** *Corynebacterium diphtheriae* reduces tellurite and produces gray-black colonies. Commensal diphtheroids are gray. *(Tellurite blood agar, 48 h at 37°C)*

Loeffler's Serum
for Corynebacterium Diphtheriae

Schick test:

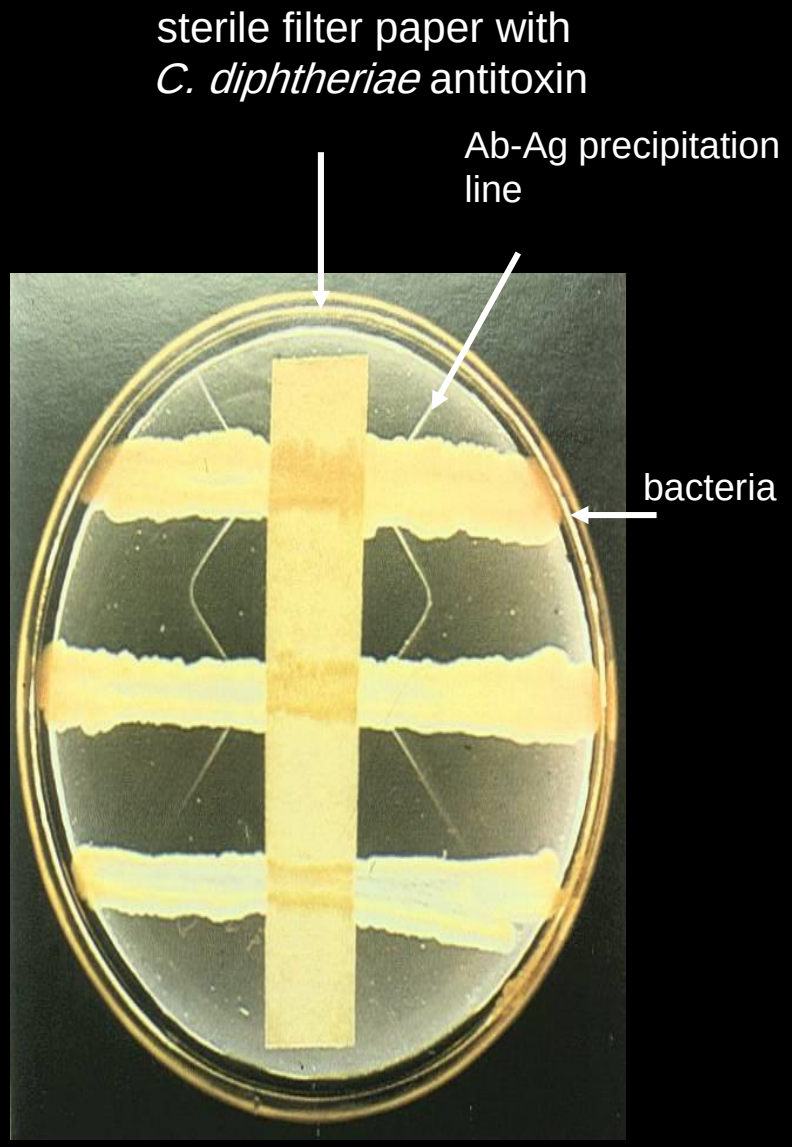
Intracutaneous skin test distinguishes between persons who are susceptible and those who are immune to diphtheria toxin and to test for sensitivity to toxoid.

Procedure: the test is performed by intradermal injection of 0.1 mL of purified standardized toxin, if the patient has no antitoxin the toxin will cause inflammation at the site of injection 4-7 days later. If no inflammation occurs, antitoxin is present and the person is immune.

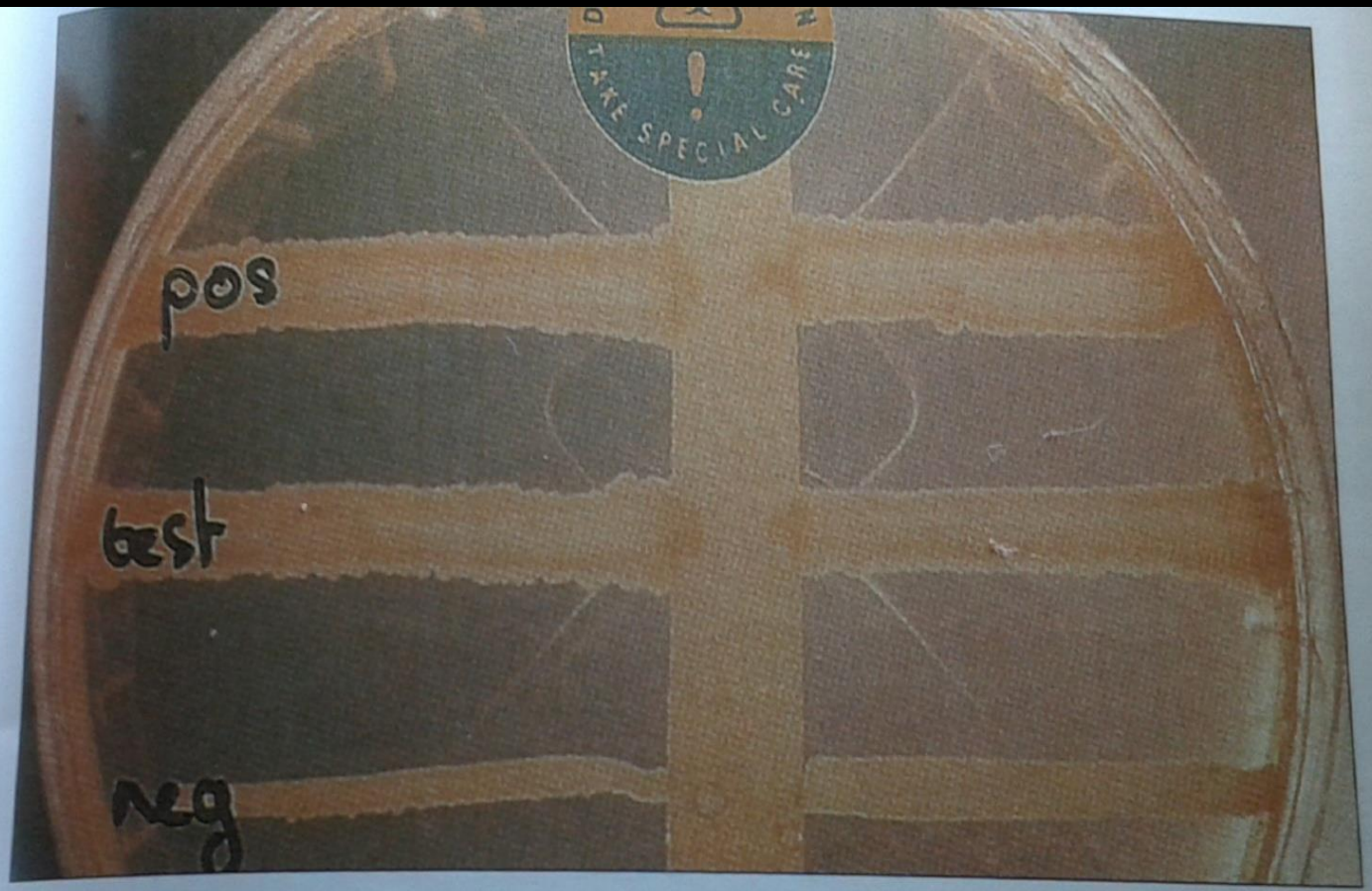
Elek's test:

In this test a strip of filter paper impregnated with diphtheria antitoxin placed on the surface of serum agar pettri dish. Narrow streaks of bacteria are made at right angles to the strip. The toxin produced by the bacteria will diffuse in the agar and where it meets antitoxin in optimum concentration will produce a line of precipitation.

- V- shaped line indicate positive result



Elek's test:



146 Elek plate to demonstrate the toxigenicity of *Corynebacterium diphtheriae*. The filter paper strip contains diphtheria antitoxin; it is placed in the Petri dish and the medium is poured on. The test strain and toxigenic and non-toxigenic strains are inoculated at right angles to the strip. A toxigenic strain produces a V-shaped line of precipitation between the toxin and anti-toxin. *(Elek's medium, 48 h at 37°C)*

Treatment and prevention:

- The treatment of choice is antitoxin. The decision to treat with antitoxin is a clinical one and cannot wait for the laboratory results.
- Penicillin G or erythromycin.

- The disease is prevented by diphtheria vaccine (DTaP).

Listeria monocytogenes:

Causes meningitis and sepsis in newborns, pregnant women, and immunocompromised patients.

Important properties:

1- small, G +ve rods arranged in L or V shape similar to *Corynebacterium*

2- ß-hemolytic

3- motile with tumbling movementat room temp.

4- grow well and multiply at refrigerator temp. (cold enhancement).

5- CAMP test +ve

Laboratory diagnosis:

- Specimens: CSF and blood
- Microscopic: Gram stain, CSF typically shows no *Listeria* because of the low bacterial concentration.
- Culture:

  Mueller-Hinton agar +sheep blood : produce a narrow zone of ß-hemolysis

*Isolation can be enhanced if the specimen is kept at 4º C for some days before inoculation into media

**Note: the motility at room temp. and the hemolysin production are primary findings that help in differentiation of *Listeria* from *Corynebacterium.*

- Identification is made by sugar fermentation tests and serology


Thank you