

Practical Medical Bacteriology

Lab 6

Laboratory Diagnosis of *Corynebacterium* spp



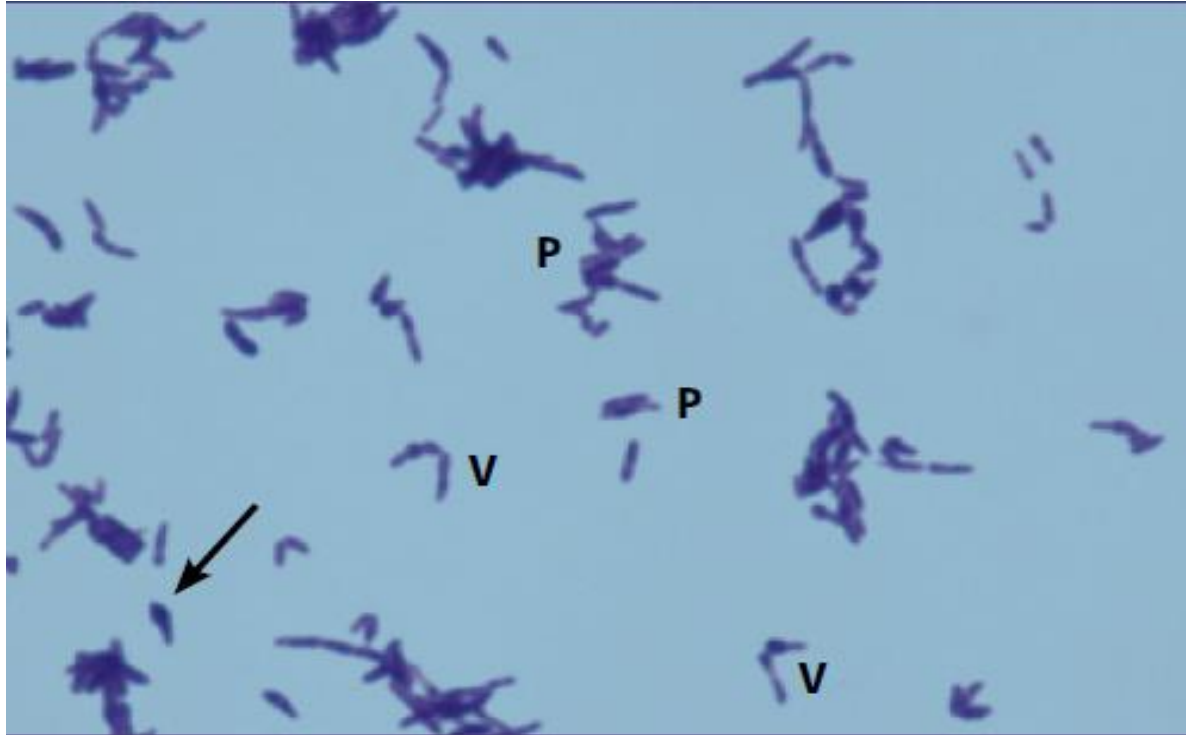
2021/2022

General features of *Corynebacteria* spp

- *Corynebacterium* spp are Gram positive, club (Coryne=club) shape arranged in single cells, in pairs (V forms), in palisades, or in clusters so called Chinese letters appearance.
- Aerobic or facultative anaerobic bacteria.
- Non-motile, non-sporulated, non-capsulated.
- Catalase positive
- Many species can be isolated from various places such as soil, water, blood, and human skin.
- *Corynebacterium diphtheriae* pathogenic species causes Diphtheria, while all other are nonpathogenic associated with human and called **diphtheroides** such as *Corynebacterium pseudodiphtheriticum*, *C. ulcerans*, *C. urealyticum* and *C. amycolatum*.



General features of *Corynebacteria* spp



Gram Stain of *Corynebacterium* spp

Laboratory diagnosis

A. Specimen: throat, nasal, wound swab.

B. Direct microscopic examination:

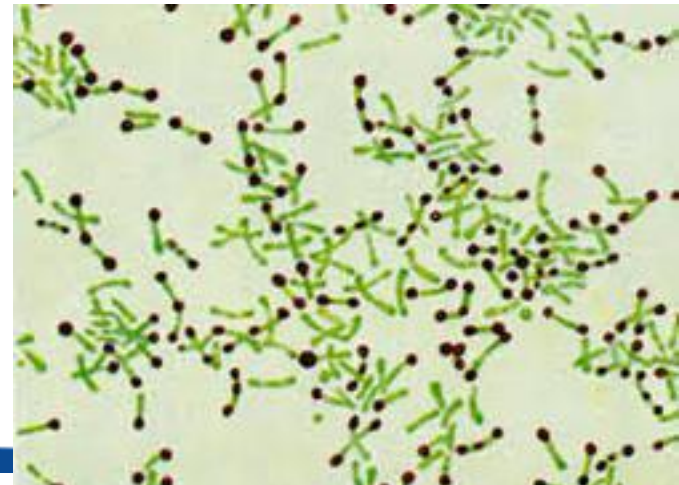
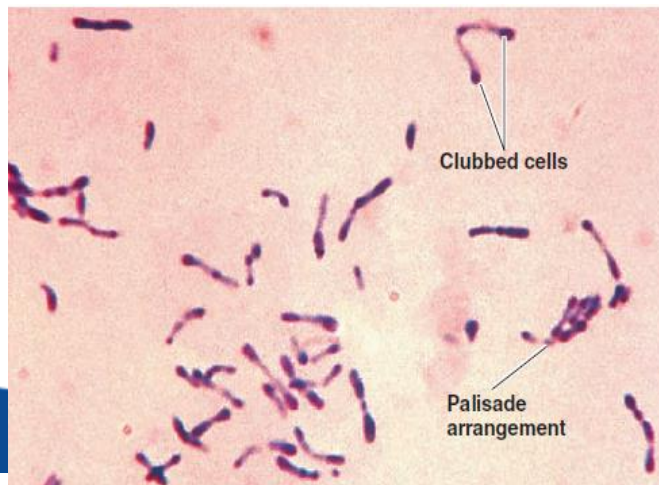
This method is commonly used for diagnosis of diphtheria plus clinical picture of the disease.

➤ **Gram stained smear from throat swab:**

Gram positive bacilli (usually slender) with Chinese letters arrangement.

➤ **Albert's stained smear from throat swab:**

Demonstrate metachromatic or volutin or Babes-Ernst granules which appear in brown color while the bacteria take greenish color.



Laboratory diagnosis

3. Cultural characteristics:

➤ On Enriched Medium

A. Blood agar: Colonies are circular and white.

B. Loeffler's serum slope: Colonies appear as circular, glistening, and white.

➤ On selective media

A. Potassium tellurite agar (PTA): *C. diphtheriae* reduces tellurite to metallic tellurium which gets incorporated into the colonies giving them black color. *C. ulcerans* and *C. pseudotuberculosis* can also producing black colored colonies.

B. Tinsdale's medium: It is a modified **PTA** added with cysteine. Black-colored colonies are produced surrounded by a brown halo, due to breakdown of cysteine by cysteinase enzyme produced by the organism.



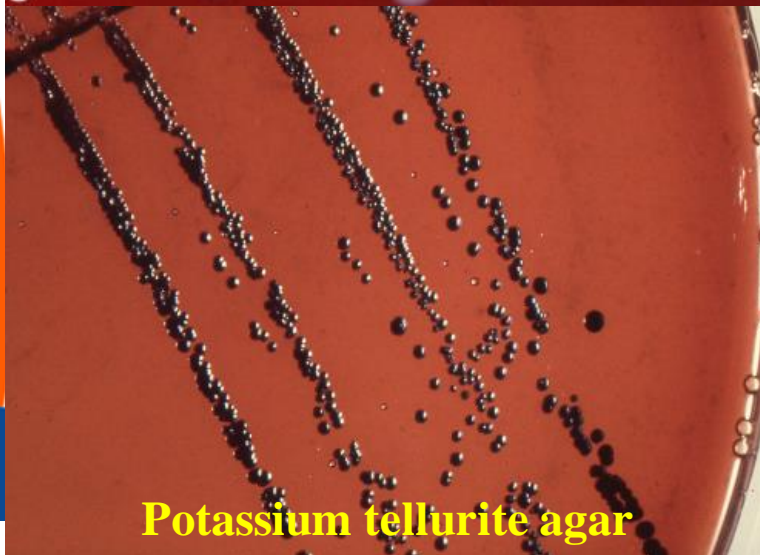
Laboratory diagnosis



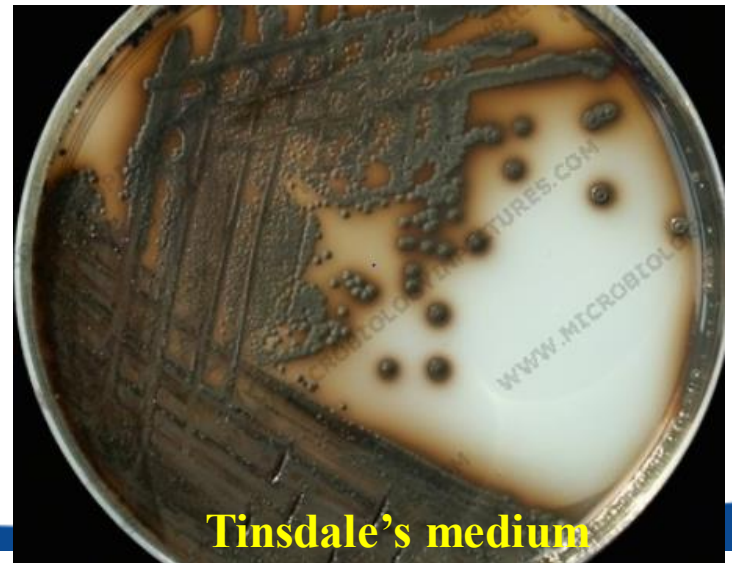
Blood agar



Loeffler's serum slope



Potassium tellurite agar



Tinsdale's medium

Laboratory diagnosis

3. Cultural characteristics:

There are 3 biotypes of *C. diphtheriae*

1. *C. diphtheriae gravis*: Large colonies responsible for severe infection.
2. *C. diphtheriae intermedius*: Intermediate colonies for moderate infection.
3. *C. diphtheriae mitis*: Small colonies responsible for mild infection.



Laboratory diagnosis

4. Preliminary tests:

- **Catalase test:** all *Corynebacterium* species are catalase positive.
- **Motility test:** all *Corynebacterium* species are non motile.

5. Biochemical tests:

- **Urease test**
 - *Corynebacterium diphtheriae*: Urease negative
 - **Diphtheroid bacilli (Normal flora):** are urease positive.



Urease test

Laboratory diagnosis

6. **Toxigenicity tests:**

- A. **Elek's test:** most common assay.
- B. **PCR:** detection of toxin gene.
- C. **ELISA:** detection of toxin from culture.
- D. **Animal inoculation:** 2 Guinea pigs one receives antitoxin as control. Diphtheria bacilli given intraperitoneally to both animals, the first not infected while the second develops infection.

Elek's test: *In vitro* detection of exotoxin

Principle:

- It is toxin/antitoxin reaction
- Toxin production by *C. diphtheriae* can be demonstrated by a precipitation between exotoxin and diphtheria antitoxin.



Laboratory diagnosis

Elek's test:

Procedure:

- A filter paper strip impregnated with diphtheria antitoxin is buried just beneath the surface of a special agar plate before the agar hardens.
- Strains to be tested and known positive and negative toxigenic strains are streaked on the agar's surface in a line across the plate and at a right angle to the antitoxin paper strip.
- After 24 hrs. of incubation at 37° C, the plates are examined with transmitted light for the presence of fine precipitin lines at a 45-degree angle to the streaks.
- The presence of precipitin lines indicates that the strain produced toxin that reacted with the homologous antitoxin.



Laboratory diagnosis

Elek's test:

Results:

- Line 1 is the negative control.
- Line 2 is the positive control.
- Line 3 is an unknown organism that is a nontoxigenic strain.
- Line 4 is an unknown organism that is a toxigenic strain.

