

بكتريا طبية 1

Gram Positive non Spore Forming Bacilli (Corynebacterium)



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قسم تقنيات المختبرات الطبية

Second Stage

المرحلة / الثانية

محاضرة رقم: 10

الجانب النظري

Lecture No. 10

Theoretical

تدريسي المادة : دكتور معين حسن

(INTRODUCTION)

- **Gram Positive non Spore Forming Bacilli**
- **Introduction**
- **Listeria**
- **Corynebacterium**

❖ **Corynebacterium diphtheriae**

□ **Introduction:**

□ **Morphology and Identification**

- Corynebacterium are 0.5–1 μm in diameter and several micrometers long.
- Characteristically, they possess irregular swellings at one end that give them the “**club-shaped**” appearance.
- Irregularly distributed within the rod (often near the poles) are granules staining deeply with aniline dyes (metachromatic granules) that give the rod a beaded appearance.
- Individual corynebacteria in stained smears tend to lie parallel or at acute angles to one another.

- True branching is rarely observed in cultures.
- On blood agar, the C diphtheriae colonies are small, granular, and gray with irregular edges and may have small zones of hemolysis.
- On agar containing potassium tellurite, the colonies are **brown to black with a brown-black halo** because the tellurite is reduced intracellularly (staphylococci and streptococci can also produce black colonies).

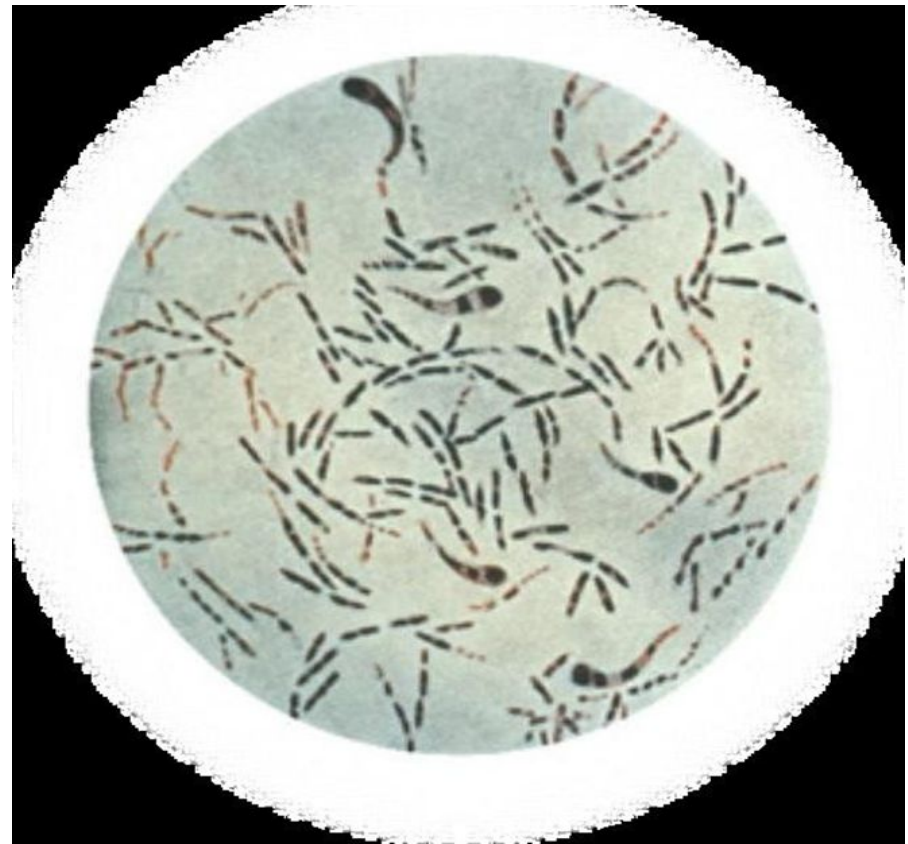
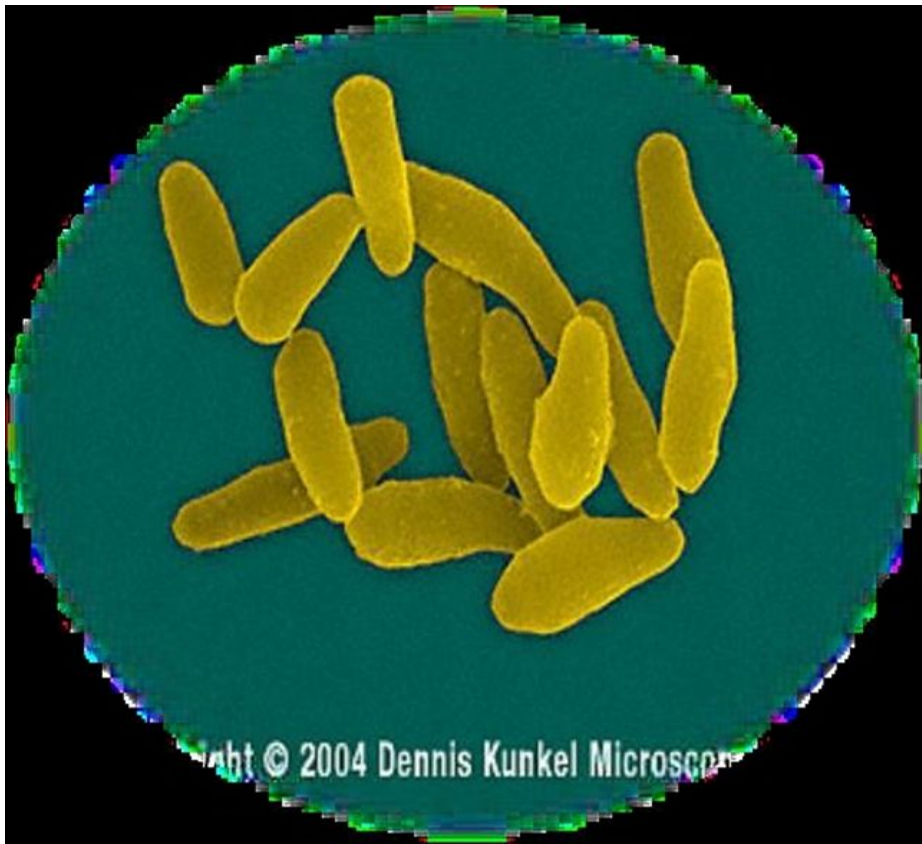
❖ Definition

- Diphtheria is an acute infectious disease that typically strikes the upper respiratory tract including the throat.
- It is caused by infection with the bacteria *Corynebacterium diphtheriae*.
- It's characterized by sore throat and mild fever at first.
- As the disease progresses, a membranous substance forms in the throat that makes it difficult to breathe and swallow.
- C. diphtheriae produces diphtheria toxin, which alters the protein function in the host by inactivating the elongation factor EF-2. As a result, this causes pharyngitis and pseudomembrane in the throat.



❖ Causes

- *Corynebacterium diphtheriae* causes diphtheria.
- Usually the bacteria multiply on or near the surface of the mucous membranes of the throat, where they cause inflammation.



- The inflammation may spread to the voice box (larynx) and may make your throat swell, narrowing your airway.
- Disease causing strains of *C. diphtheriae* release a damaging substance (toxin), which can also involve the heart, brain and nerves.



❑ Pathogenesis and Virulence Factors.

❑ Virulence Factors.

- Diphtheria toxin is the major virulence factor associated with *C. diphtheriae*.
- This toxin is produced by strains of *C. diphtheriae* infected with a lysogenic β -phage, which carries the tox gene for diphtheria toxin.
- Nontoxigenic strains can be converted to tox⁺ by infection with the appropriate β -phage.
- Only toxin-producing *C. diphtheriae* causes diphtheria; however, *C. ulcerans* and *C. pseudotuberculosis*, which belong to the “*C. diphtheriae* group,” can also produce the toxin when they become infected with the tox-carrying β -phage.
- Diphtheria toxin is a protein of 62,000 Da.
- It is composed of two fragments, A and B, which are linked together by a disulfide bridge.

- The toxin is exceedingly potent and is lethal for humans in amounts of 130

- **The toxicity is due** to the ability of diphtheria toxin to **block protein synthesis** in eukaryotic cells.
- The toxin is secreted by the bacterial cell and is nontoxic until exposed to trypsin.
- Trypsinization **cleaves** the toxin into the two fragments.
- Both fragments are necessary for cytotoxicity.
- **Fragment A** is responsible for the cytotoxicity, and fragment B binds to receptors on the eukaryotic cells and mediates the entry of fragment A into the cytoplasm.
- On reaching the cytoplasm, fragment A **disrupts** protein synthesis.
- Fragment A **splits nicotinamide adenosine dinucleotide** to form nicotinamide and adenosine diphosphoribose (ADPR).
- ADPR binds to and inactivates elongation factor 2 (EF-2), an enzyme

- Production of the toxin in vitro depends on numerous environmental conditions, including an alkaline pH (7.8 to 8.0), oxygen, and, most importantly, the iron concentration in the environment.
- The amount of iron needed for optimal toxin production is less than the amount needed for optimal growth.
- The toxin is released in significant amounts only when the available iron in the culture medium is exhausted.

❖ **Diphtheria Exotoxin**

- It is a heat labile polypeptide composed of two fragments :
A (active) and B (binding).
- Virulence is due to an exotoxin
- It inhibits protein synthesis - kills cells
- Causes necrosis (death) of cells in upper respiratory tract -
Pseudomembrane formation.
- Toxin can be distributed systemically to cause life threatening
damage to organs

❖ **Signs and symptoms**

- A sore throat
- Painful swallowing
- Swollen glands (enlarged lymph nodes) in your neck
- Signs and symptoms usually begin two to five days after a person
becomes infected

❑ Laboratory Diagnosis

- Dacron swabs from the nose, throat, or other suspected Specimens should be inoculated to a blood agar plate (to rule out hemolytic streptococci). and a selective medium such as a **tellurite plate** (e.g., cystine-tellurite blood agar [CTBA] or modified Tinsdale's medium) and incubated at 37°C in 5% CO₂.
- Plates should be examined in 18–24 hours.
- In 36–48 hours, the colonies on **tellurite medium** are sufficiently definite for recognition of C diphtheriae.
- On cystine tellurite agar, the colonies are **black with a brown halo**.
- A presumptive C diphtheriae isolate should be subjected to testing for toxigenicity.
- Such tests are performed only in reference public health laboratories.

1. Staining methods

- Grams method
- Albert's stain
- Neisser's stain
- Ponders stain
- On staining seen as:

Pairs, Appear as V and L letters, resembling Chinese letter pattern or also called cuneiform arrangement.

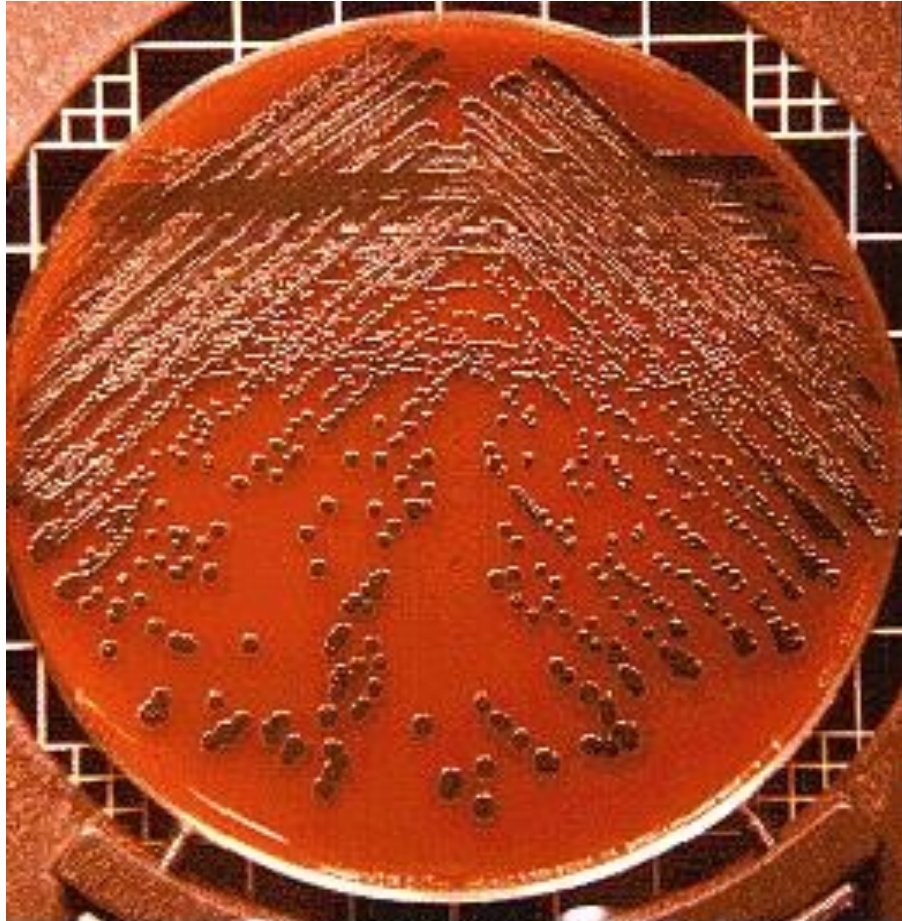
2. Cultural characters

- Need **enrichment Media** contain (Blood, Serum or Egg, 37 c and ph. 7.4).
- Aerobic/Facultative anaerobic.

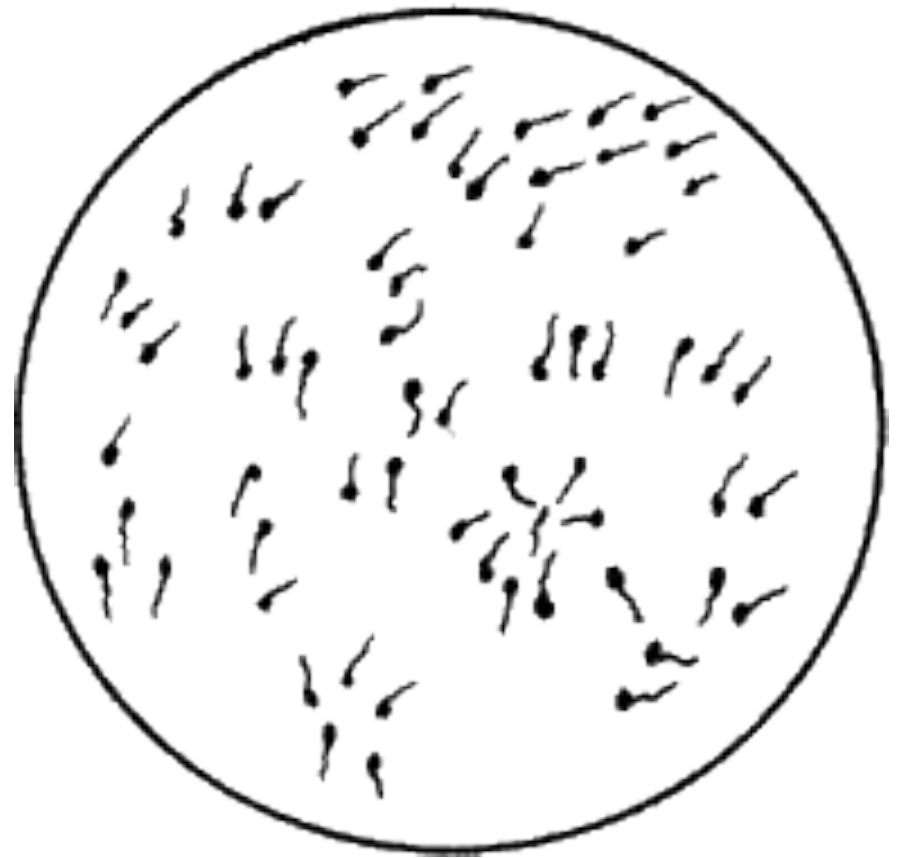
□ Commonly used medium.

- Loeffler serum slope.
- Tellurite Blood agar.

Gram +ve Bacilli and Colonies

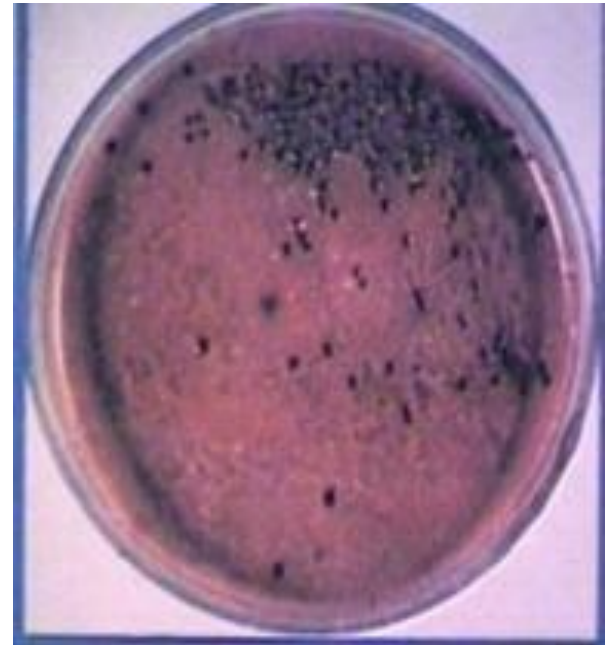


***Corynebacterium diphtheriae*, mitis**
Chocolate tellurite agar



Culturing

- Selective & differential medium
- Corynebacterium are resistant to tellurite
 - Reduced to tellurium
- Forms deposit in colonies
 - Colonies appear dark
- Biotypes
 - gravis, Intermedius, mitis



Growing on Culture Plates

- Loffler serum slope
Grows rapidly in
6 -8 hours,
Small white opaque
disks
Turns to yellow
Tellurite blood
agar
Modified Mac Leod
Hoyles medium.



Commonly used medium

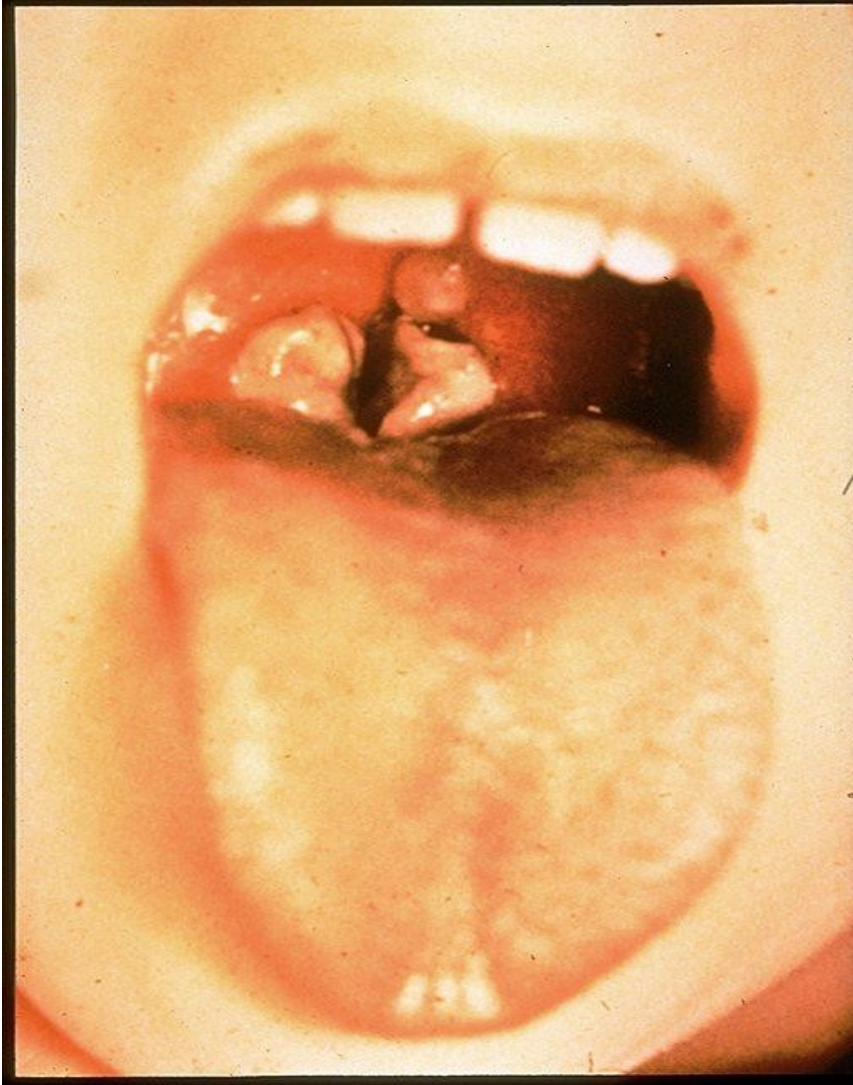
- Tellurite blood agar
Contains tellurite
0.04 tellurite Inhibits
other bacteria
- Produce
Grey/Black
colonies.



Thick Membrane



Pseudo membrane



3. Modified Elek immunoprecipitation method

- Described by the World Health Organization Diphtheria Reference Unit.
- A filter paper disk containing antitoxin (10 IU/disk) is placed on an agar plate.
- The cultures to be tested (at least 10 colonies should be chosen) for toxigenicity are spot inoculated 7–9 mm away from the disk.
- After 48 hours of incubation, the antitoxin diffusing from the paper disk has precipitated the toxin diffusing from toxigenic cultures and has resulted in precipitin bands between the disk and the bacterial growth.

4. Polymerase chain reaction (PCR) based methods

- Have been described for detection of the diphtheria toxin gene (tox).
- PCR assays for tox can also be used directly on patient specimens before culture results are available.
- A positive culture result confirms a positive PCR assay.
- A negative culture result after antibiotic therapy along with a positive PCR assay result suggests that the patient probably has diphtheria.

5. Enzyme-linked immunosorbent assays

- Can be used to detect diphtheria toxin from clinical *C diphtheriae* isolates.

6. An immunochromatographic strip assay

❑ Treatment and vaccination

- The treatment of diphtheria rests largely on rapid suppression of toxin-producing bacteria by antimicrobial drugs and the early administration of specific antitoxin against the toxin formed by the organisms at their site of entry and multiplication.
- Antimicrobial drugs (**penicillin, macrolides**) inhibit the growth of diphtheria bacilli.
- Although these drugs have virtually no effect on the disease process, they arrest toxin production and assist public health efforts.
- They also help to eliminate coexistent streptococci and C diphtheriae from the respiratory tracts of patients or carriers.
- **DPT** is a class of combination vaccines against three infectious diseases in humans: diphtheria , pertussis (whooping cough), and tetanus.

QUIZ

Q1/ Describe the clinical infections associated with *Corynebacterium diphtheriae*.

Q2/ Discuss several nondiphtheria *Corynebacterium* spp. that are capable of causing clinical infection in humans.

Q3/ Discuss *Corynebacterium diphtheriae* Virulence Factors (Diphtheria toxin).

Q4/ 1. Skin diphtheria as occurs in children in tropical areas typically:

- (A) Does not occur in children who have been immunized with diphtheria toxoid
- (B) Is clinically distinct from skin infections (pyoderma, impetigo) caused by *Streptococcus pyogenes* and *Staphylococcus aureus*
- (C) Is also common in northern latitudes
- (D) Results in protective antitoxin levels in most children by the time they are 6–8 years old

QUIZ

2- All of the following statements regarding *Corynebacterium diphtheriae* are true, except?

- A. It can be identified by using tests for toxigenicity
- B. The toxin inhibits protein synthesis
- C. Toxin has adverse effects on cardiovascular and nervous systems
- D. Native chromosome is responsible for toxin production

Correct answer: D.

3- Elek's gel precipitation test is for

- A- Gonococcus
- B- Diphtheria
- C- Hemophilus

QUIZ

4- Diphtheria toxin acts by

- A- Inhibiting acetylcholine release
- B- Inhibiting glucose transport
- C- Increasing levels of cyclic AMP
- D- Inhibiting protein synthesis

Correct answer: D.

5- One of the following statements regarding diphtheria toxin is true:

- A- Toxin is produced by all corynebacteria
- B- Positive Schick test suggest immunity
- C- Toxin production is influenced by critical concentration of iron
- D- It is an endotoxin

Any questions?

*Thank
You*