Corynebacterium diphtheriae

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Corynebacterium-Introduction

- Corynebacteria :
  - are Gram-positive, aerobic, nonmotile, rod-shaped bacteria classified as **Actinobacteria**.
  - They do not form spores or branch as do the actinomycetes, but they have the characteristic of forming irregular, club-shaped or V-shaped arrangements in normal growth.
  - They undergo snapping movements just after cell division, which brings them into characteristic forms resembling Chinese letters or palisades.

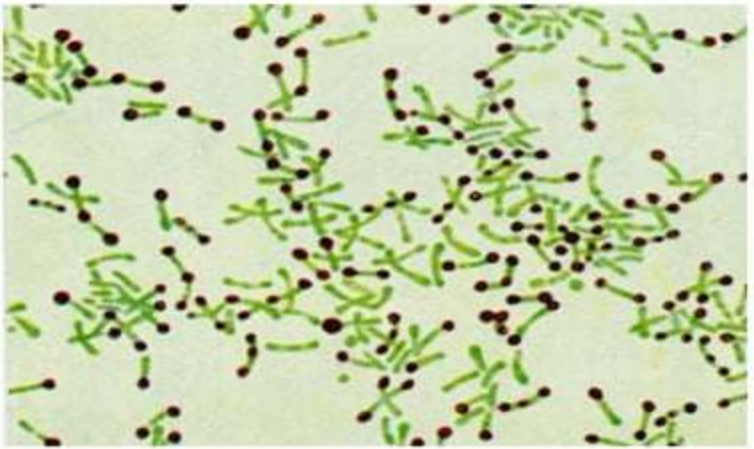
Corynebacterium-Introduction

- The genus *Corynebacterium* consists of a diverse group of bacteria including :
  - Some are saprophytic
  - Some produce disease in animals.
  - Some corynebacteria are part of the normal flora of humans, finding a suitable niche in virtually every anatomic site, especially the skin and nares.
  - *C. diphtheriae* is the most important pathogen in the group. The best known and most widely studied species is ***Corynebacterium diphtheriae***, the causal agent of the disease **diphtheria**.

Corynebacterium diphtheriae

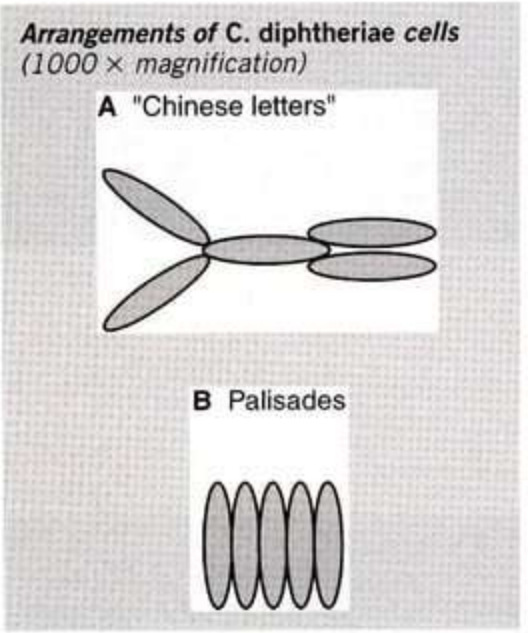
- Morphology:
  - *Corynebacterium diphtheriae* is a nonmotile, noncapsulated, club-shaped, Gram-positive rod shaped bacillus.
  - 0.5–1 m in diameter and several micrometers long.
  - Possess iregular swellings at one end that given them the "club shaped"apperance.
  - Metachromic granules are distributed within the rod which ives a beaded appearance.
  - Tend to lie parallel or at acute angles to one another.

Diphtheroids Gram stain



Stained *Corynebacterium* cells. The "barred" appearance is due to the presence of polyphosphate inclusions called metachromatic granules. Note also the characteristic "Chinese-letter" arrangement of cells.

Arrangement of *C. diphtheria*



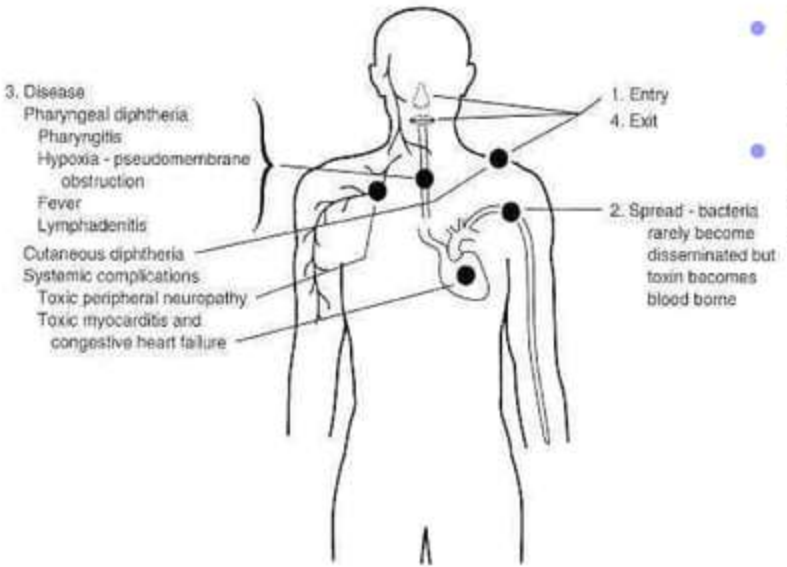
C. diphtheria-Identification

- 4 morphological types of *C. diphtheriae* are found on tellurite containing media:
  - Mitis – black colonies with a gray periphery
  - Gravis – large, gray colonies
  - Intermedius – small, dull gray to black.
  - Belfanti
  - All produce an immunologically identical toxin.
  - In general var gravis tends to produce more severe disease than var mitis,but similar illness can be produced by all types
- Incubation -35-37^{0} C for 24 hours.
- They prefer a pH of 7.8-8.0 for good growth.
- They require access to oxygen (poor AnO_2 growth).

C. diphtheria-pathogenesis

- The pathogenesis of diphtheria is based upon two primary determinants:
  - (1) the ability of a given strain of *C diphtheriae* to colonize in the nasopharyngeal cavity and/or on the skin, and
  - (2) its ability to produce diphtheria toxin.
- Since those determinants involved in colonization of the host are encoded by the bacteria, and the toxin is encoded by the corynebacteriophage, the molecular basis of virulence in *C diphtheriae* results from the combined effects of determinants carried on two genomes. however, they may become highly virulent following lysogenic conversion to toxigenicity.

C. diphtheria-pathogenesis



- **Early stages:** Sore throat. Low fever. Swollen neck glands.
- **Late stages:** Airway obstruction and breathing difficulty. Shock

C.diphtheriae-Toxins

- Virulence factors- *C. diphtheriae*
  - For *C. diphtherias* to cause diphtheria an exotoxin must be produced.
    - Is a heat-labile polypeptide produced during lysogeny of a β phage that carries the "tox" gene.
    - Alkaline pH of 7.8- 8.0, aerobic conditions, and a low environmental iron level are essential for toxin production (occurs late in the growth of the organism).
    - The toxin inhibits protein synthesis by ADP-ribosylating elongation factor 2.

C.diphtheriae-Toxins

- Trypsin cleaves the toxin into 2 fragments, A and B, that are linked together by a disulfide bridge.
- Fragment B is required for toxin binding to tissue cells and fragment A contains the toxic activity.
- Fragment A is the N-terminal 21 kDa component of the toxin and contains the catalytic center for the ADP-ribosylation of elongation factor 2 (EF-2) according to the following reaction:

$$NAD^+ + EF\text{-}2 \rightarrow ADPR\text{-}EF\text{-}2 + H^+.$$

- One molecule of toxin can inhibit 90% of the protein synthesis in a cell.
- Systemic effects include heart failure, paralysis and adrenal hypofunction leading to an Addison's like disease.

C. diphtheria toxin

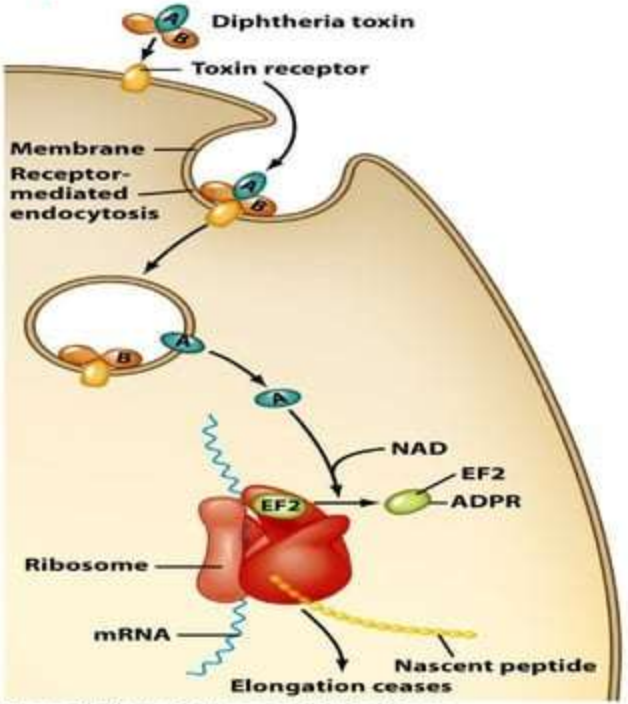
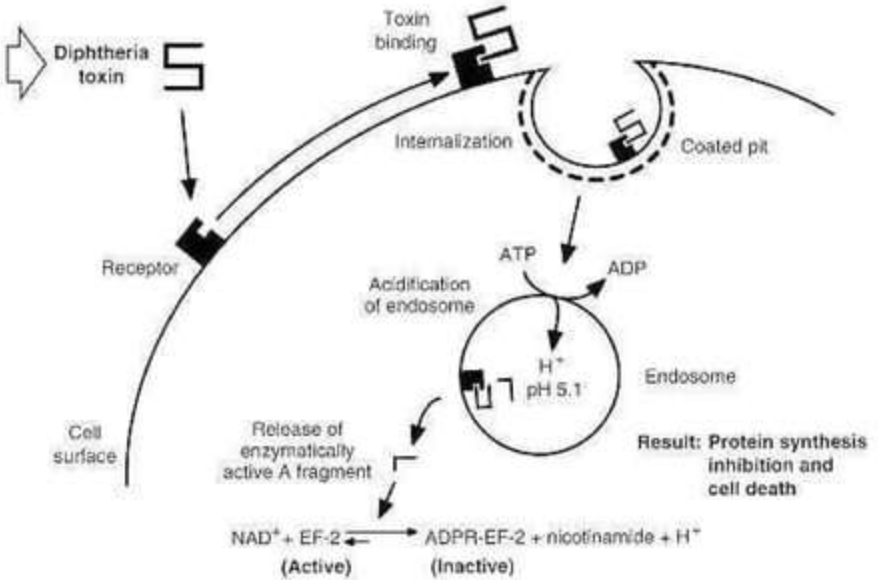


Figure 25.22 Microbiology: An Evolving Science

- Toxin enters through receptor mediated endocytosis
- Acidification of endocytic vesicle allows A to dissociate from B
- A enters cycoplasm

C. diphtheria toxin



C. diphtheria toxin

- The intoxication of a single eukaryotic cell by diphtheria toxin involves at least four distinct steps:
  - (1) the binding of the toxin to its cell surface receptor;
  - (2) clustering of charged receptors into coated pits and internalization of the toxin by receptor-mediated endocytosis; following acidification of the endocytic vesicle by a membrane-associated, ATP-driven proton pump,
  - (3) the insertion of the transmembrane domain into the membrane and the facilitated delivery of the catalytic domain to the cytosol,
  - (4) the ADP-ribosylation of EF-2, which results in the irreversible inhibition of protein synthesis. It has been shown that a single molecule of the catalytic domain delivered to the cytosol is sufficient to be lethal for the cell.

C.diphtheriae-Diagonosis

- The clinical diagnosis of diphtheria requires bacteriologic laboratory confirmation of toxigenic *C diphtheriae* in throat or lesion cultures.
- For primary isolation, a variety of media may be used:
  - Loeffler agar, Mueller-Miller tellurite agar, or Tinsdale tellurite agar.
  - Sterile cotton-tipped applicators are used to swab the pharyngeal tonsils or their beds.
  - Calcium alginate swabs may be inserted through both nares to collect nasopharyngeal samples for culture.
  - Since diphtheritic lesions are often covered with a pseudomembrane, the surface of the lesion may have to be carefully exposed before swabbing with the applicator.

C.diphtheriae-Diagonosis

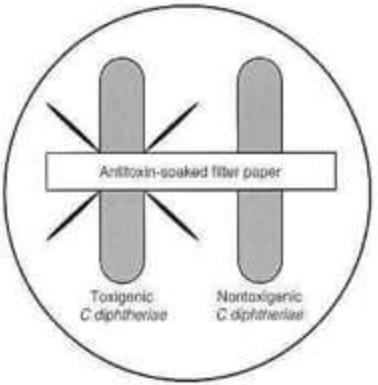
- The toxigenicity of *C diphtheriae* strains is determined by a variety of *in vitro* and *in vivo* tests.
- The most common *in vitro* assay for toxigenicity are:
  - the Elek immunodiffusion test
  - Polymerase chain reaction-detection of the diphtheria toxin gene *(tox)*.
  - Enzyme-linked immunosorbent assays -detect diphtheria toxin from clinical *C diphtheriae* isolates.
  - (4) An immunochromographic strip assay -detection of diphtheria toxin in a matter of hours. This assay is highly sensitive.

C.diphtheriae-Diagonosis

- To prove that an isolate can cause diphtheria, one must demonstrate toxin production.
  - This is most often done on an Elek plate:
    - The organism is streaked on a plate containing low iron.
    - A filter strip containing anti-toxin antibody is placed perpendicular to the streak of the organism.
    - Diffusion of the antibody into the medium and secretion of the toxin into the medium occur.
    - At the zone of equivalence, a precipitate will form.

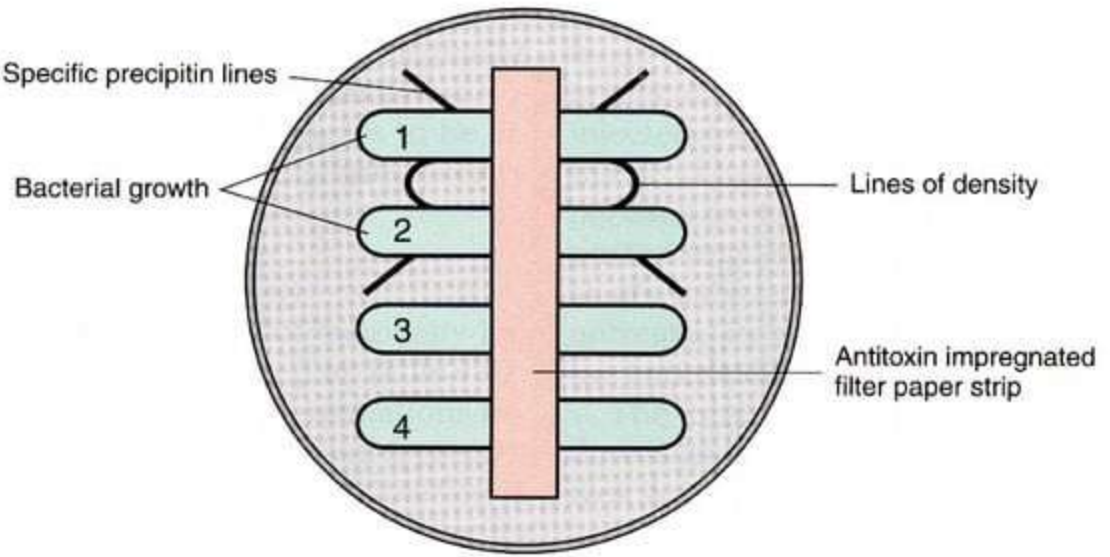
Elek immunodiffusion test

- A sterile, antitoxin-saturated filter paper strip is embedded in the culture medium, and *C diphtheriae* isolates are streak-inoculated at a 90° angle to the filter paper.
- The production of diphtheria toxin can be detected within 18 to 48 hours by the formation of a toxin-antitoxin precipitin band in the agar.



Sterile filter paper impregnated with diphtheria antitoxin is imbedded in agar culture medium. Isolates of *C diphtheriae* are then streaked across the plate at an angle of 90° to the antitoxin strip. Toxigenic *C diphtheriae* is detected because secreted toxin diffuses from the area of growth and reacts with antitoxin to form lines of precipitin.

Elek plate


Specific precipitin lines
Bacterial growth
1
2
3
4
Lines of density
Antitoxin impregnated
filter paper strip

C.diphtheria-Clinical manifestation.

- Clinical Significance (*C. diphtheria*)
  - Is normally found in the throats of healthy carriers.
    - The organism infects only man and it has a limited capacity to invade.
  - Diphtheria - Disease usually starts as a local infection of the mucous membranes causing a membranous pharyngitis
    - Local toxin effects result in degeneration of epithelial cells.
    - Inflammation, edema, and production of a pseudomembrane composed of fibrin clots, leukocytes, and dead epithelial cells and microorganisms occurs in the throat.

Diphtheria - pseudomembrane

- This may obstruct the airway and result in suffocation.

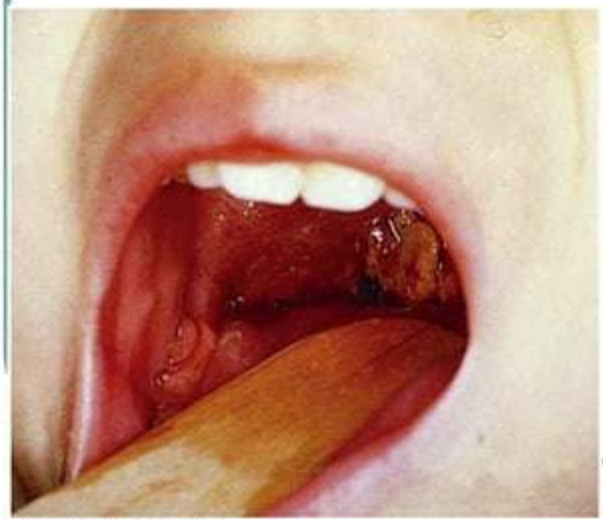


Figure 25.20b Microbiology: An Evolving Science
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C.diphtheriae-other complications

- The more dangerous effects occur when the toxin becomes systemic and attacks the heart (heart failure), peripheral nerves (paralysis), and the adrenal glands (hypofunction).
- Cutaneous diphtheria- More common in tropical and subtropical areas.
  - Necrotic lesions with occasional formation of a local pseudomembrane occur.

- Antibiotic susceptibility and treatment
  - Antiserum - once the toxin has bound, however, the antiserum against it is ineffective.
  - Penicillin- to eliminate the organism.

Treatment and Control

- Prevention- Active immunization with toxoid (alum precipitate).
  - Is part of the DPT vaccine.
- Shick skin test- like the Dick test in that it tests for circulating antibody to the toxin by injecting a small amount of toxin intradermally and observing for a local erythematous and necrotic reaction.
  - If this occurs it indicates that the person has no anti-toxin antibodies and is, therefore, susceptible to diphtheria.
- Other Corynebacterium- are part of the normal flora of the skin and URT.

<<<QUESTION PLS>>>