



# CORYNEBACTERIUM

## 19.1 INTRODUCTION

*Corynebacterium diphtheriae* is a pathogenic bacterium that causes diphtheria. It is also known as the Klebs-Löffler bacillus, because it was discovered in 1884 by German bacteriologists Edwin Klebs (1834 – 1912) and Friedrich Löffler (1852 – 1915).



## OBJECTIVES

After reading this lesson, you will be able to:

- describe the morphological characteristics of the *Corynebacterium diphtheriae*
- explain the clinical features of diphtheria
- discuss the laboratory diagnosis of *Corynebacterium diphtheriae*
- explain the disease spectrum caused by *Corynebacterium diphtheriae*

## 19.1 MORPHOLOGY

Corynebacteria are gram – positive , non- acid fast , nonmotile rods with irregularly stained segments , and sometimes granules. They frequently shows club shaped swelling and hence the names corynebacteria( from *coryne*, meaning club). The most important member of this genus is *C diphtheria* sp, the causative agent of diphtheria.

### ***Corynebacterium diphtheriae***

Humans are the sole pathogen reservoir for diphtheria. Infection sources include infected persons and carriers (rare). The disease is usually transmitted by droplet

## Corynebacterium

infection, or less frequent indirectly via contaminated objects. It is an acute and **contagious infection characterized by pseudomembranes** of dead epithelial cells, white blood cells, red blood cells, and fibrin **that form around the tonsils and back of the throat**. It is an uncommon illness that tends to occur **in unvaccinated individuals**, especially school-aged children, those in developing countries, elderly, neutropenic or immunocompromised patients. The virulent and toxigenic strains are lysogenic, and produce an exotoxin formed by two polypeptide chains, which is itself produced when a bacterium is transformed by a gene from the  $\beta$  prophage.

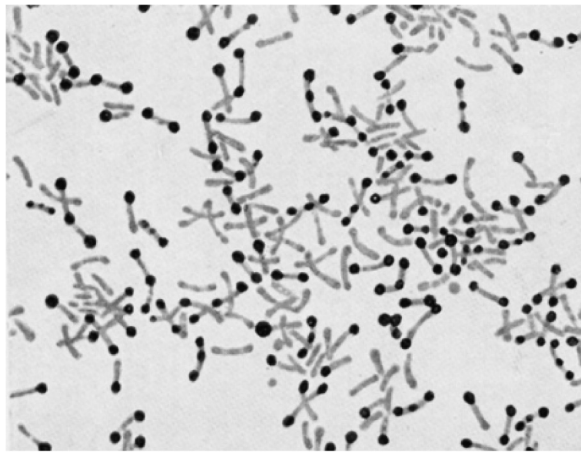


Fig. 19.1

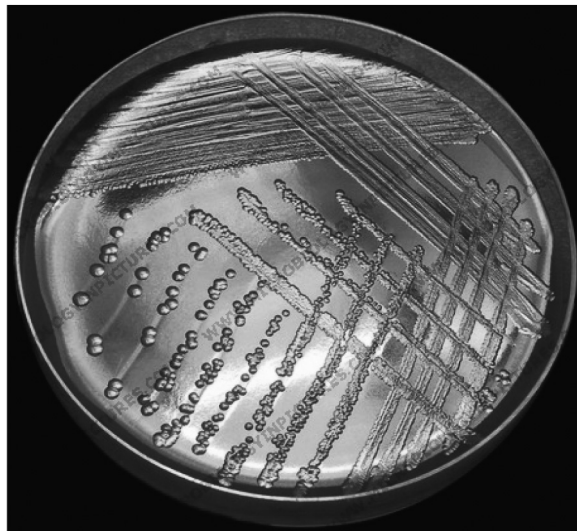


Fig. 19.2: *Corynebacterium diphtheriae*

## MODULE

Microbiology



Notes



## Notes

Four subspecies are recognized: *C. diphtheriae mitis*, *C. diphtheriae intermedius*, *C. diphtheriae gravis*, and *C. diphtheriae belfanti*. The four subspecies differ slightly in their colonial morphology and biochemical properties such as the ability to metabolize certain nutrients, but **all may be toxigenic** (and therefore cause diphtheria) **or non-toxigenic**. Unusually, the diphtheria toxin gene is actually encoded by a bacteriophage which is found in toxigenic strains, not on the bacterial chromosome itself.

### Clinical Features

- **Respiratory:** Following an incubation period of 2-4 days, patients typically report upper respiratory tract symptoms (eg, nasal discharge, sore throat). The posterior pharynx and tonsillar pillars are most often involved. Onset is often sudden, with low-grade fevers, malaise, and membrane development on one or both tonsils, with extension to other parts of the respiratory system.
- **Cardiac:** The toxic effect in the myocardium characteristically occurs within 1-2 weeks following onset of infection, often when the upper respiratory tract symptoms are improving. Manifestations are due to arrhythmias and congestive heart failure (CHF).
- **Neurologic:** Neurological symptoms can occur immediately or after several weeks. Bulbar symptoms generally occur within the first 2 weeks after disease onset and can range from mild symptoms (eg, difficulty swallowing) to bilateral symmetric paresis of the palatal and ocular muscles. The bulbar symptoms may remit or progress to paralysis of the proximal and then distal skeletal muscles over the next 30-90 days. Although recovery can be very slow, patients generally regain complete neurologic function. Secondary complications include aspiration from bulbar paralysis and bronchopneumonia from respiratory muscle dysfunction.
- **Skin:** Cutaneous infections can occur, often in more tropical climates, presenting as nonhealing ulcers. A recent surveillance study of Native Americans presenting to the Indian Health Service clinics in South Dakota recovered *C diphtheriae* from 6 (5%) of the 133 patients, 1 of whom had skin ulcers.

### Laboratory Diagnosis

Consists of isolation of the diphtheria bacilli and demonstration of its toxicity.

Samples – two swabs from the lesion are collected under vision

Microscopy – perform gram stain and albert staining

## Corynebacterium

Gram stain – The bacilli is a slender rod with tendency to clubbing at one or both the ends. The bacilli are pleomorphic. They are nonsporing, noncapsulated and nonmotile. They are gram positive but tends to decolourised easily. The granules are composed of polymetaphosphate granules which are more gram positive from rest of the bacteria.

Albert stain – green colour bacilli are seen with black colour granules.

Morphology – the bacilli is a slender rod with tendency to clubbing at one or both ends. The bacilli are pleomorphic. They are nonsporing, noncapsulated and nonmotile. They are gram positive but tends to decolourised easily. The granules are composed of polymataphosphate granules which are more gram positive from rest of the bacteria.



Fig. 19.3: Gram staining

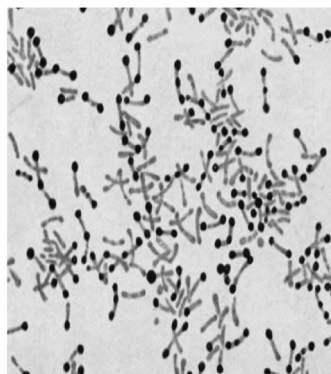


Fig. 19.4: Albert staining of *Corynebacterium diphtheriae*

### Cultural Characteristics

Growth is scanty ordinary media. Enrichment with blood, serum or egg is necessary for good growth. The optimum temperature for growth is 37°C (range 15 - 40°C) and the optimum pH is 7.2. It is an aerobe and a facultative anaerobe.

The usual media employed for the cultivation of the diphtheria bacillus are Loeffler's serum slope and tellurite blood agar. Diphtheria bacilli grow on

## MODULE

Microbiology



Notes

## MODULE

Microbiology



### Notes

## Corynebacterium

Loeffler's serum slope very rapidly and colonies can be seen in 6-8 hours, long before other bacteria grow. Colonies are at first small, circular white opaque discs but enlarge on continued incubation and may acquire a distinct yellow tint.

Diphtheria bacilli ferment with the production of acid, (but not gas) glucose, galactose, maltose and dextrin (but not lactose, mannitol or sucrose). Some strains of virulent diphtheria bacilli have been found to ferment sucrose. It is necessary to use Hiss's serum water for testing sugar fermentation. Proteolytic activity is absent. They do not hydrolyse urea or form phosphatase.

### Toxin

Virulent strains of diphtheria bacilli produce a very powerful toxin. The pathogenic effect of the bacilli are due to toxin. Almost all strain of gravis and intermedius (about 95 – 99 percent) are toxigenic while only about 80 – 85 per cent of the mitis strains are so.

Diphtheria toxin is a protein. It has two fragments, A and B. Both the fragments are necessary for toxic effect. When released by the bacterium, the toxin is inactive as on fragment A is masked. All the enzymatic activity of the toxin is present in fragment A. Fragment B is responsible for the binding of the toxin to the cell.

### Virulence Test

Virulence test – any isolate of the diphtheria bacilli should be tested for virulence or toxigenicity for the bacteriological diagnosis to be complete. Virulence testing may be by vivo or invitro methods, the former by the subcutaneous or intradermal test and the latter by the precipitation test or the tissue culture test. In vivo tests are done on guinea pig.

### Invitro Test

Invitro test- Elek's gel precipitation test : A rectangular strip of filter paper impregnated with diphtheria antitoxin (1000 units/ml) is placed on the surface of a 20% normal horse serum agar in a petri dish while the medium is still fluid. When the agar is set, the surface is dried and narrow streaks of the strain are made at right angle to the filter paper strip. A positive and negative control should be put up. The plate is incubated at 37°C for 24 – 48 hours. Toxin produced by the bacterial growth will diffuse in the agar and where it meets the antitoxin of optimum concentration, will produce a line of precipitation. The presence of arrow head lines of precipitates indicates that the strain is toxigenic. No precipitate will form in the case of nontoxigenic strains.

### Tissue Culture Test

Tissue culture test : The toxigenicity of diphtheria bacilli can be demonstrated by incorporating the strains in the agar overlay of the cell culture monolayers. The toxin produced diffuses into the cells below and kills them.

### Prophylaxis

Diphtheria can be controlled by immunization . Diphtheria toxoid is usually given in children as a trivalent preparation containing tetanus toxoid and pertussis vaccine also., as a DPT, DPT or triple vaccine

### Sensitivity

The bacterium is sensitive to the majority of antibiotics, such as the penicillins, ampicillin, cephalosporins, quinolones, chloramphenicol, tetracyclines, cefuroxime and trimethoprim.

### Diphtheroides

Corynebacterium resembling *C.diphtheriae* occurs as normal commensals in the throat, skin, conjunctiva and other areas. These may sometimes be mistaken for diphtheria bacilli and are diphtheroids. In general they stain more uniformly than diphtheria bacilli, possess few or no metachromatic granules and tend to be arranged in parallel rows (palisade) rather than cuneiform pattern.



Notes



### INTEXT QUESTIONS 19.1

1. Which of the following is not the staining method of corynebacterium
  - (a) Gram stain
  - (b) Albert stain
  - (c) Ponders stain
  - (d) Ziehl Neelsen stain
2. What is selective media for growth of corynebacterium diphtheria
  - (a) Potassium tellurite blood agar
  - (b) Loweinstein Jenson medium
  - (c) Sabourds Dextrose agar
  - (d) Maconky 's agar
3. Corynbacteium are ..... shaped
4. Causative agent of dipheteria is .....
5. Diphtheria is transmitted by ..... infection
6. Culturally the bacteria is .....



Notes

**WHAT YOU HAVE LEARNT**

- Corynebacterium is a Gram-positive, rod-shaped bacteria, frequently shows club shaped swelling and hence the name Coryne meaning club
- Four Subspecies are recognized *C.diphtheriae mitis*, *C.diphtheriae intermedius*, *C.diphtheriae gravis* and *C. diphtheriae belfanti*.
- *C. diphtheriae* sp causes diphtheria, an acute and contagious form which is transmitted by droplet infection
- Diphtheria occurs in unvaccinated individuals especially school-aged children.
- Laboratory diagnosis consists of isolation of diphtheria bacilli and demonstration of its toxicity
- For Microscopic examination gram staining and Albert staining
- Diphtheria can be controlled by immunization of DPT vaccine
- Diphtheroides resembles corynebacterium occurring as normal commensals in throat, skin, conjunctiva

**TERMINAL QUESTIONS**

1. Clinical features of diphtheria?
2. Laboratory diagnosis of corynebacterium diphtheria?
3. Short note on diphtheroides?

**ANSWER TO INTEXT QUESTIONS**

1. (d)
2. (a)
3. Club
4. *C.diphtheria*
5. Droplet
6. Pleomorphic