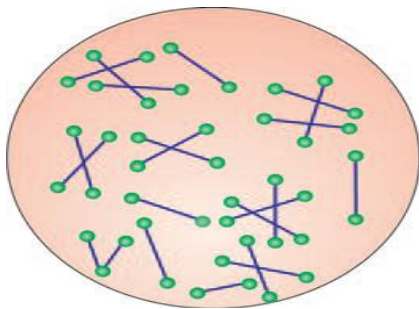


# *Corynebacterium*

## *Corynebacterium diphtheria*

**Morphology:** It is thin, slender, rod 3 to 6  $\mu \times$  0.6 to 0.8  $\mu$  showing clubbing at one or both ends. It is non-sporing, non-capsulated and non-motile. It is Gram-positive and shows pleomorphism. The presence of metachromatic granules (Babes-Ernst granules) serve to distinguish it from diphtheroid. The granules are colored dark purple with methylene blue, Albert or Neisser's differential stain.

The bacilli are arranged in characteristic fashion. It is seen in pairs or groups. Bacilli form various angles with each other like V or L. This is called Chinese letter arrangement.



**Cultural character:** Enrichment of media with blood, serum or egg is necessary for good growth.

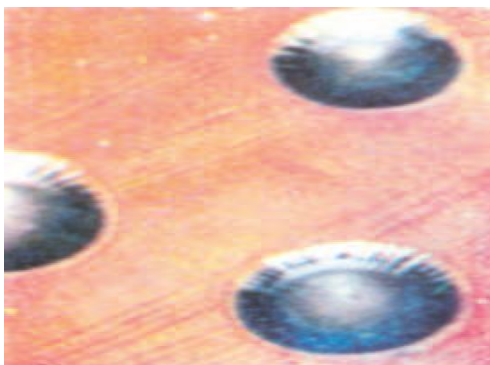
The optimum temperature is 37°C and pH 7.2. It is aerobic and facultative anaerobic.

1. *Serum broth*: Turbidity, pellicle formation, amount and nature of deposit is useful in identification of types of *Corynebacterium diphtheriae*.

2. Loeffler's slope culture shows abundant growth after 6 to 8 hours incubation. The colony is small, granular, moist, creamy and glistening with irregular edges.

3. *Blood tellurite medium* (0.04%): It is useful in differentiation of *Corynebacterium diphtheriae* into gravis, mitis and intermedius types. It acts as selective media inhibiting the growth of other organisms.

Diphtheria bacilli reduce tellurite to metallic tellurium giving gray to black color to colonies.



## Biochemical Reactions

It ferments glucose and maltose with acid production only. Fermentation of starch, glycogen and dextrin is useful for recognition of gravis, intermedius and mitis. Gravis ferments starch, glycogen and dextrin while intermedius and mitis have no action on starch and glycogen.

It is catalase positive, oxidase negative and do not liquefy gelatin. Urea is not hydrolyzed.

It is indole negative and do not form phosphatase.

## Complications

1. Asphyxia – a mechanical obstruction by pseudomembrane.
2. Acute circulatory failure which may be peripheral or central.
3. Post diphtheritic paralysis, e.g. palatine, ciliary, occurring in 3rd or 4th week of disease with spontaneous recovery.
4. Septic, e.g. pneumonia and otitis media.

## Laboratory Diagnosis

*Hematological investigations:*

It is not significant.

## Bacteriological Investigations

Laboratory confirmation of diphtheria is necessary for control measures and epidemiological purposes.

Specific treatment should be given immediately without waiting for laboratory test report.

1. *Smear examination:* Gram-staining shows thin, Gram-positive bacilli showing Chinese letter arrangement. Albert staining is done for the demonstration of metachromatic granules .

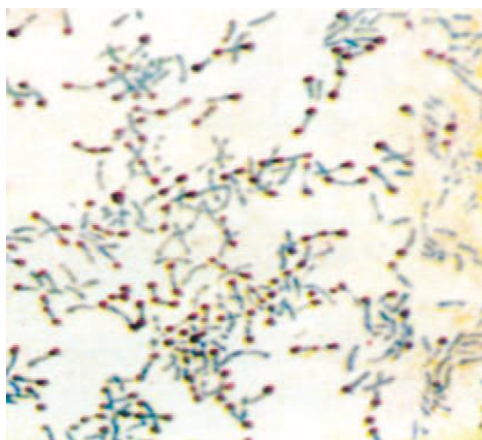
2. *Culture:* The swab is inoculated on Loeffler's slope, blood tellurite media and blood agar plate. The serum slope shows growth in 6 to 8 hours. Smear is stained with Albert stain and we may find bacilli with metachromatic granules and typical arrangement. Blood tellurite plate may be incubated for at least 2 days before declaring it negative.

Individual strain of *C. diphtheriae* within a biotype can be identified by phage typing

*In vitro:* The gel precipitation test is called Elek's test. A rectangular strip of filter paper impregnated with diphtheria antitoxin (1000 unit/ml) is placed on the surface of 20 percent normal horse serum agar in petridish while medium is still in fluid form. When it dries, testing strain is inoculated at right angle to filter paper strip. This plate is inoculated at 30°C for 24 to 48 hours. Toxin produced by bacterial growth will diffuse in agar and where it meets optimum concentration will produce line of precipitation. No precipitate will occur in non toxigenic strain.

*Tissue culture test:* This may be done by incorporation of *Corynebacterium diphtheriae* into an agar overlay of cell culture monolayers. Toxin produced may diffuse into cells and kills them.

Primary isolates can be rapidly screened for toxigenicity by PCR.



## ***Bacillus***

**General characters:** They are rod shaped, sporogenous classified into two groups:

- a. Aerobic bacillus.
- b. Anaerobic clostridia

Aerobic bacilli are Gram-positive, nonmotile, spore bearing bacilli occurring in chains.

They are thick with truncated or convex ends. They include psychrophilic, mesophilic and thermophilic species. The salt tolerance varies from less than 2 to 25 percent of NaCl.

*Bacillus anthracis* is the only pathogenic species causing anthrax whereas *B. subtilis* is opportunist and *B. cereus* may produce food poisoning.

It remains in parasitic form in cattle and sheep. Infection in man is the result of accidental contact with infected animal.

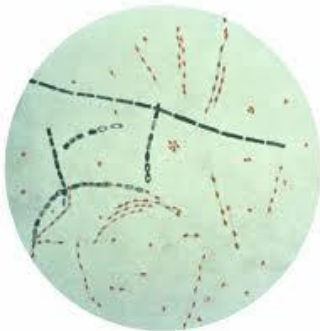
**Morphology:** It is non-motile, non-acid fast, Gram-positive measuring  $1 \times 3$  to  $4 \mu$ . They may be arranged singly or in short chains. The entire chain may be surrounded by capsule.

Capsule is polypeptide in nature (D-glutamic acid).

Capsule production depends upon a 60 megadalton plasmid, p × O<sub>2</sub>. In culture the bacilli are arranged end to end in chains. The chain of bacilli presents bamboo stick appearance. Spores are formed in soil only in presence of oxygen and not in animal body.

Sporulation may be brought about by:

1. Distilled water.
2. 2 percent NaCl.
3. Growth on oxalated agar shows spores



### **Cultural characters:**

It is aerobic growing at optimum temperature of 37°C (range being 12°C to 45°C). The optimum temperature for spore formation is 25°C to 30°C. Growth may occur on ordinary media.

a. *Nutrient broth*: There may be floccular turbidity or no turbidity.

b. *Agar plate*: Colony is irregular, around 2 to 3 mm in diameter, raised, dull opaque, greyish white with a frosted glass appearance and cut glass appearance (in transmitted light). With magnifying glass they look like tangled mass of long hair like curls (barrister wig or medusa head appearance) .

Virulent capsulated strain forms rough colonies whereas a virulent forms smooth colonies.

c. *Blood agar*: The colony is non-hemolytic.

d. *Gelatin stab*: A characteristic “inverted tree” appearance is seen with slow liquefaction starting from the top as shown in

e. *Selective medium (PLET)*: It consists of polymyxin, lysozyme, ethylene diamine tetra acetic acid (EDTA) and thallous acetate added to heart infusion agar. It is used to isolate anthrax from mixture of spore bearing bacilli.



Medusa head appearance of colony on  
tree

nutrient agar  
appearance



Gelatin stab showing inverted

### Biochemical Reactions

Glucose, maltose and sucrose are fermented with acid production only. Nitrates are reduced to nitrite, catalase is positive and gelatin is liquefied.

### Laboratory Diagnosis

#### A. Hematological investigations:

Leukocytosis occurs when tissues are invaded otherwise total leukocyte count is within normal limit.

#### B. Bacteriological investigations:

1. *Microscopic examination*: Smear prepared from exudate, sputum, etc. on Gram's staining shows Gram-positive non-sporeing bacilli occurring in chain.
2. *Culture*: The material is inoculated on nutrient agar plate. Smear shows Grampositive spore bearing bacilli.
3. *Animal inoculation*: A small amount of exudate or isolated culture from infected man is injected subcutaneously in guinea pig. Guinea pig dies within 36 to 48 hours. Smear from heart blood and spleen shows typical Gram-positive bacilli.
4. *Serological test*: It is a precipitation test. It is used in making rapid diagnosis. The infected tissues are grounded in saline, boiled for 5 minutes and filtered. This tissue extract is layered over anthrax antiserum. Zone of precipitate at the junction of tissue extract and antiserum within 5 minutes at room temperature means test is positive. It is called ASCOLI TEST.
5. *McFadyean reaction*: When blood films containing anthrax bacilli are stained with polychrome methylene blue for a few seconds and examined under the microscope, amorphous purplish material is seen around bacilli. This represents capsular material and is characteristic of anthrax bacilli.
6. *Polymerase chain reaction*: Using this technique *Bacillus anthracis* may be confirmed.

Bicarbonate agar and blood agar  
plate cultures of *Bacillus anthracis*

