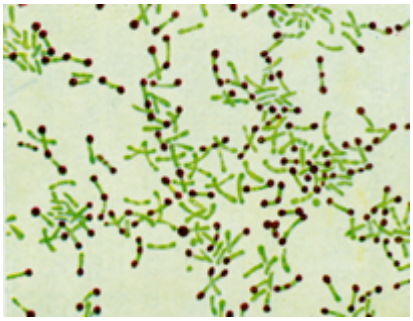


Corynebacterium

Koryne = club: bacterium = organism

Corynebacteria are 0.5–1 ⌠m in diameter and several micrometers long. Characteristically, they possess irregu- lar swellings at one end that give them the "club-shaped" appearance, non motile , gram positive catalase (+) , no gas in carbohydrate medium , pathogenic species produce power full exotoxin, non spore formed non capsulated .

Type species**:** *corynebacterium diphtheriae*

Common names = diphtheria bacillus , klebs — loeffler bacilli K LB.

Klebs in 1883 observed and described diphtheria bacillus from pseudomembranes from the throat patients. loeffler in 1884 , suggested its etiological relationship to disease and isolated diphtheria bacilli in pure culture and produced lesions in animals. The bacilli are highly pleomorphic and arranged in angled pairs parallel rows (palisades) looking like Chinese letters character. Some may be seen as L or V forms. The diphtheria bacillus exhibits marked tendency to stain irregularly resulting in a beaded appearance. These deeply stained bands have been variously named as metachromatic granules, babes Ernst or volutin granules and are mainly composed of high molecular weight (polymerized) polyphosphate. They may be present at one end or both ends of the cell or scattered with in the cell substance with Albert staining, volutin granules are stained deep blue and the cytoplasm greenish.

Cultural character: aerobic bacteria, optimum growth temperature 37°C an alkaline reaction in a pH range of 7.8-8 is required for primary isolation, diphtheria bacillus is best cultured on enriched media , common media employed for primary isolation are :

1) loeffler's serum medium
2) blood agar
3) blood tellurite medium

Loeffler's serum medium : growth on this medium is rapid and minute colonies appear after 8-24h incubation much before the other bacteria grow.

Morphology of the bacteria is characteristic when stained smears from colonies on this medium are examined.

Blood tellurite medium:

Three varieties of diphtheria bacillus, the gravis intermedius and mitis are recognized on this medium . it has been known that potassium tellurite in amounts that inhibit the growth of most bacteria has little effect on C. *diphtheriae* and this provides a selective means for isolate and diagnosis (tellurite reduction occurs with in the bacteria after the tellurite passes the cell membrane , it is reduced to the metal tellurium) giving grey or black color to colonies the gravis causing the most severe and the mitis the mild variety with the intermedius being responsible for diseases of intermediate severity . The gravis and intermedius types are associated with high case fatality rate , whereas mitis infections are less lethal .

Biochemical reaction: *C.diphtheriae* ferment with acid production glucose galactose , maltose and dextrin, it does not ferment lactose , sucrose, mannitol and does not liquenfy gelatin .**H_2S(+),** catalase**(+).**

Diphtheria toxin: Toxigenicity is under control of the phage gene, virulence is under the control of bacterial gene . Production of toxin is markedly influenced by environment and nutritive conditions even strongly toxigenic strains may produce little or no toxin under unfavorable conditions a slight alkaline reaction (ph 7.8-8.0) is essential. Free access of air is essential peptone is important for the production of toxin, the optimum concentration of iron being 0.14 g/ml.

Nature of toxin:

It is highly lethal for certain animals. Two distinct substances designated (A) and (B) enter into its constitution and toxins from different strains may very in relative amount of these constitution . The toxin is heat labile protein and can be converted to toxoid by formalin its toxic action is believed to be due to competitive inhibition of the mammalian respiratory enzymes, cytochrome C. the toxin has special affinity for myocardium, adrenal tissue and nerve endings the toxin is released extracellular as single polypeptide chain with a molecular weight of about 62000 Dalton, it consists of two fragments **A** and B both of these fragments are required for a toxic effect in animals and tissue culture cells .all the enzyme activity of the toxin resides in fragment A. This fragment alone unable to enter the cell with out the hydrophobic fragment B, which provides a mechanism for the transport and attachment of fragment A to sensitive sites on the cell membrane. Fragment B has no independent activity. The toxin is lethal for man, rabbits , guinea pigs and birds .**rate and mice are highly resistant because their cells lack binding sites for fragment B.**

Tests for toxigenicity: It is important to determine whether the isolated strain is toxigenic or not. The toxigenicity tests may be performed by:

In vivo intradermal two animal tests. Sub cutaneous single animal test (Guinea pigs or rabbit)

In vitro gel precipitation test

In vitro gel precipitation test : (Elek's test): Eleks medium contained horse serum is used . plase over the surface of the medium , a sterile filter paper strip 60X15 mm previously soaked in diphtheria antitoxin 1000 units /ml , and excess drained off. The surface of the medium is dried for about 30 minutes in the incubator before in inoculation.

Inoculate the plate by heavy inoculum of the diphtheria bacilli to be tested, across the plate at right angles to the filter paper strip. A known positive and negative controls are included more than one strain can be inoculated at the same time. Incubate and examine after 24h to 48h for the line of precipitate, which will appear about half a centimeter away from the filter strip, and an angle to the line of inoculation the antitoxin diffuses out far filter strip and toxin diffuses side away from the line of inoculation and growth and where they meet a optimum neutralizing proportions, a line of precipitation is formed.

Pathogenicity for man:

The incubation period in diphtheria is commonly 3-4 days, but may be as short as one day, in man diphtheria is usually a local infection of the mucous membrane and the pharynx is most commonly effected , but infection of the larynx and nasal diphtheria are not infrequently observed growth of diphtheria bacilli initiated by desquamation of epithelial cells of the superficial layer of mucous membrane by small amount of toxin .

This toxin is observed into the adjoining living cells, destroys them in a few hours through its local necrotic action, the nidus of necrotic tissue supplies favorable ground for further growth of the organisms and more toxin is formed and the process extends, profuse fibrinous exudation and characteristic thick, grayish smelly false membrane containing fibrin , dead tissue cells , leucocytes and large No. of diphtheria bacilli is formed. At first this membrane is grayish, but it soon become thick and tough forming a dull white layer or pseudo membrane covering the area. The initial lesion may cover tonsil and then extend further and patient may show manifestations of toxemia, with relatively low fever with temperature 38°C to 39°C laryngeal involvement it may result in mechanical stoppage of the air passage and death of the patient.

Schick test: In 1913 schick described test based on the fact that when a minute amount of diphtheria toxin is introduced intradermal it exerts a local destructive or necrotic effect on the cells of the skin and the underling tissue, if the blood passing through the tissue contains sufficient antitoxin , 1/500 to 1/250 or more of a unit of antitoxin per ml , the injected toxin is neutralized and thus no reaction occurs . The reaction in susceptible person having less than certain amount of the antitoxin in the blood , show visible local reaction . this reaction has been widely applied with a view Of gauging immunity or susceptibility to diphtheria .

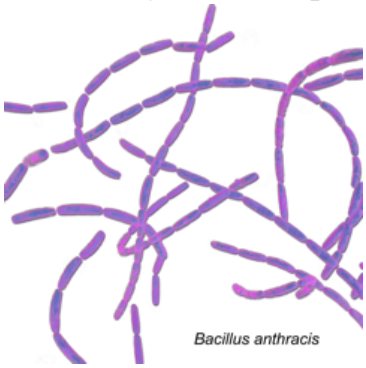
Laboratory diagnosis: Preferably Sterile swabs are taken from the site of lesion , often rubbing over the affected area and if possible the portion of the pseudo membrane may be removed the various methods employed for the bacteriological diagnosis includes .

1-Smear examination: smear for light microscope, fluorescent antibody microscope

2-cultural examination

3-pathogenicity test.

Bacillus anthracis



Gram positive bacteria, non motile, straight or slightly curved rods, Measuring 4-10 urn length and 1-1.5 urn is width , it is one of the largest pathogenic bacteria in tissue it is found singly , in pairs or short chains but under artificial culture variable length chains are present (capsule is formed during growth in the animal body and can be demonstrated in blood and animal tissue . the capsular material is a high molecular weight polypeptide of D-glutamic acid the spores are found in culture , is soil and material from dead animal , but not in living animal tissue . the spores are central , elliptical and not bulging .

Mcfadyean reaction: This staining reaction with polychrome methylene blue has been used in veterinary practice for the recognition of anthrax bacilli in blood of infected animals, the blood film is dried and passed rapidly three time through the flame and then stained with polychrome methylene blue for a few second, washed, dried and examined. The presence of an amorphous purplish material around the bacilli representing disintegrate capsules of the organisms is characteristic of the anthrax bacillus.

Cultural characters: The bacteria is aerobic, facultative an aerobe, optimum growth Temperature being 37°C at pH 7.5-7.8 the bacteria grow readily on ordinary culture media and the growth is not much improved by the

addition of blood serum or glucose. It can grows in nutrient broth and on nutrient agar the colonies after 24h are about 2-3mm in diameter, irregular in outline opaque dull, grayish white, surface is irregular giving roughish appearance and emulsifies with difficulty. The colonies are **(medusa head)**. On blood agar usually there is (no haemolysis) around the colonies, thus differentiating anthrax bacillus from saprophytic members, which are markedly lytic.

PLET medium: A selective medium consisting of polymyxin , lysozyme, EDTA, (ethylene diamine tetra acetic acid) and thalous acetate , added to heart infusion agar , has been devised to isolate *Bacillus anthracis* from contaminated material .

Biochemical reactions: These are not of much practical value the identification and classification of the anthrax bacillus .The bacteria ferments glucose maltose sucrose salicin dextrin with the production of acid and no gas. Catalase (+) serological test are not widely employed in the identification of the bacteria except **Ascoli precipitation test** used in diagnosis of anthrax.

Pathogenesis: In man the infection is acquired from animal sources. Three portals of entry have been recognized:

A) Through damaged skin (cutaneous anthrax)
B) Through mucous membranes (gastrointestinal anthrax)
C) Through respiratory tract (Inhalation anthrax)

This bacteria cause lesion known as malignant pustule. Infection may occur by intestinal route also it may occur by inhalation of spores carried in the dust of filaments of wool In wool factories when it is referred as wool sorters disease .

Toxins : Anthrax toxin is a complex of three fractions that act synergistically they - have been named as :

1)The protective antigen (a protein)
2)The oedema factor (EF)
3)The lethal Factor (LF)

Individually the are not toxic , but the whole complex produces local oedema , shock due to increased capillary permeability .

Ascoli precipitin test:

It is used for recognition of anthrax infection in organs and tissues from suspected animals, about 2 gm of the tissue is boiled, for 5 min with 5 ml of normal saline to which acetic and has been added in the proportion of 1:1000. The fluid is filtered. 0.5 ml of anthrax antiserum is placed in a narrow tube and clear filtrate is carefully on the top of serum. The development of white ring of precipitate at the junction of the two fluids with in 10 minutes at room temperature denotes a positive result.