Corynebacterium

Gram-positive bacilli

Non–spore-forming

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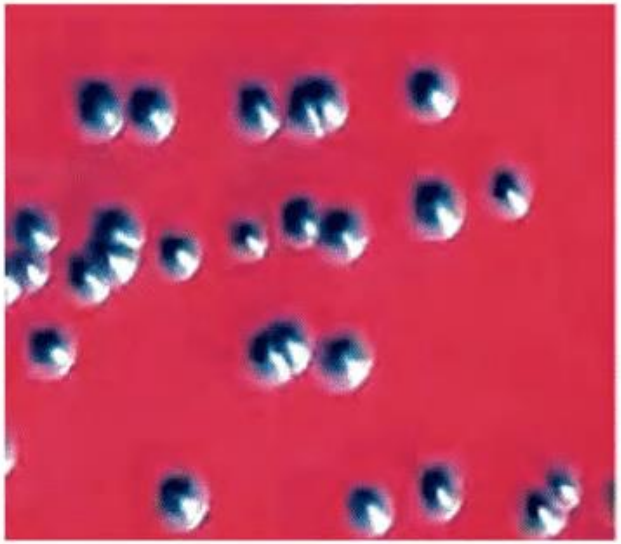
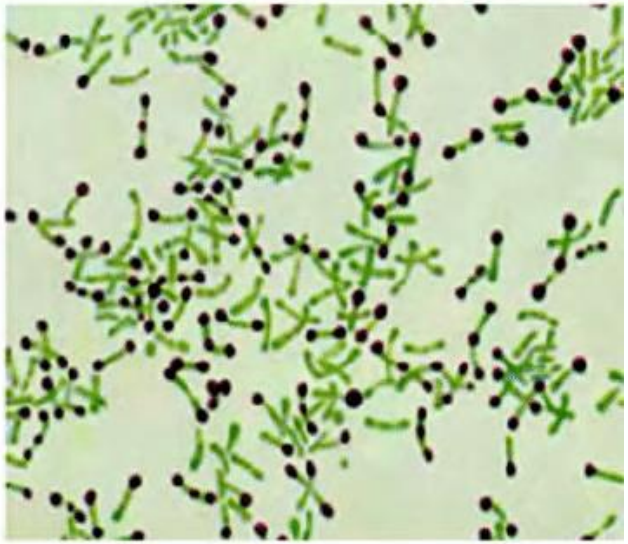
General characteristics

- Non-spore-forming, gram-positive bacilli
- Normal flora of skin and mucous membranes of humans and, as such, are frequently contaminants of clinical specimens submitted for diagnostic evaluation
- Produces a powerful exotoxin that causes diphtheria in humans, patients

General characteristics

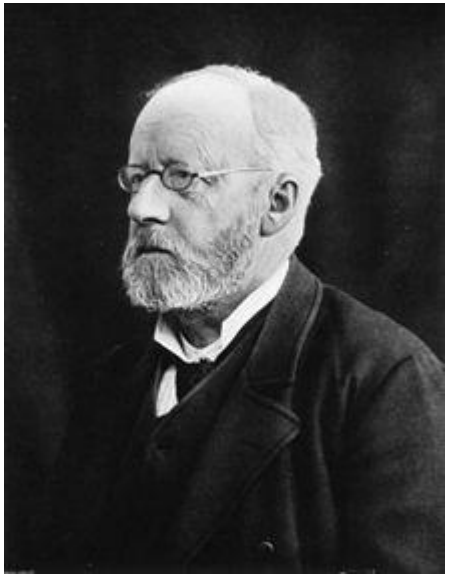
- **Corynebacterium species tend to be clubbed or irregularly shaped; although not all isolates have the irregular shapes, the terms coryneform or diphtheroid bacteria are convenient ones for denoting this broad group**





Morphology and Identification

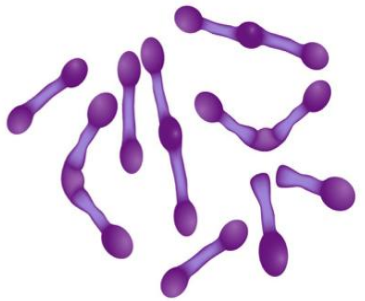
Corynebacterium diphtheriae



- From Greek: diphthera = leather
- Diphtheria bacillus , Klebs-Löffler bacillus (K LB)
- Klebs in 1883 observed and described diphtheria bacillus from pseudomembranes from the throat patients
- Löffler in 1884, suggested its a etiological relationship to disease and isolated diphtheria bacilli in pure culture and produced lesions in animals

Morphology and Identification

- Corynebacteria are 0.5–1 µm in diameter and several micrometers long
- They possess irregular swellings at one ends that give them the “club-shaped” Appearance (Figure)
- Non motile, no capsule
- Catalase positive, no gas
- Irregularly distributed within the rod



Corynebacterium diphtheriae

Morphology and Identification

- 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.
- Individual corynebacteria in stained smears tend to lie parallel (palisades) or at acute angles to one another
- The bacilli are highly pleomorphic looking like Chinese letters character, some may be seen as L or V forms.

Diagnostic Laboratory Tests

- C. diphtheriae and other corynebacteria grow aerobically on most ordinary laboratory media.
- Optimum growth temperature 37C an alkaline reaction in the pH range of 7.8-8,0 is required for primary isolation, diphtheria bacillus is best cultured on enriched media
- Common media employed for primary isolation:
- Löffler's serum medium
- Blood agar
- Blood tellurite medium

Diagnostic Laboratory Tests

Löffler's serum medium

- On Löffler serum medium, corynebacteria grow much more readily than other respiratory organisms
- Corynebacteria tend to pleomorphism in microscopic and colonial morphology that characterized them
- Growth on this medium is rapid and minute colonies appear after 8-24hrs
- Incubation much before the other bacteria grow

Diagnostic Laboratory Tests

- the Löffler's medium is nutritionally inadequate
- For the diphtheria bacillus, unbalanced cell wall synthesis Causes the organism to become pleomorphic and assume their easily recognizable morphology)
- Colonies on this medium are small, opaque ,white circular disks with regular border, later the border becomes crenated and the center becomes elevated and yellow in color.

Diagnostic Laboratory Tests

Blood agar

On blood agar, the C. diphtheriae colonies are small, granular, and gray with irregular edges and may have small zones of hemolysis.

- Four biotypes of C diphtheriae have been widely recognized: gravis, mitis, intermedius, and belfanti.
- These variants have been classified on the basis of growth characteristics such as colony morphology, biochemical reactions, and severity of disease produced by infection.

Diagnostic Laboratory Tests

Blood tellurite medium

- 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).
- Three varieties of diphtheria bacillus, the gravis intermedius and mitis are recognized on this medium

Diagnostic Laboratory Tests

- 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, mitis the 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 .

Diagnostic Laboratory Tests

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

- **Smear examination**

Smear for light microscope, fluorescent antibody microscope

- **Cultural examination**
- **Pathogenicity test**

Biochemical reaction

- C. diphtheriae ferment glucose, galactose, maltose and dextrin with acid production.
- It does not ferment lactose, sucrose, mannitol
- Does not liquefy gelatin
- H2S(+)
- 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 μg/ml.**

- When some nontoxigenic diphtheria organisms are infected with bacteriophage from certain toxigenic diphtheria bacilli
- The offspring of the exposed bacteria are lysogenic and toxigenic, and this trait is subsequently hereditary.
- When toxigenic diphtheria bacilli are serially subculture in specific antiserum against the temperate phage that they carry, they tend to become nontoxigenic.
- Whereas toxigenicity is under control of the phage gene, invasiveness is under control of bacterial genes.

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 extracellularly 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 .

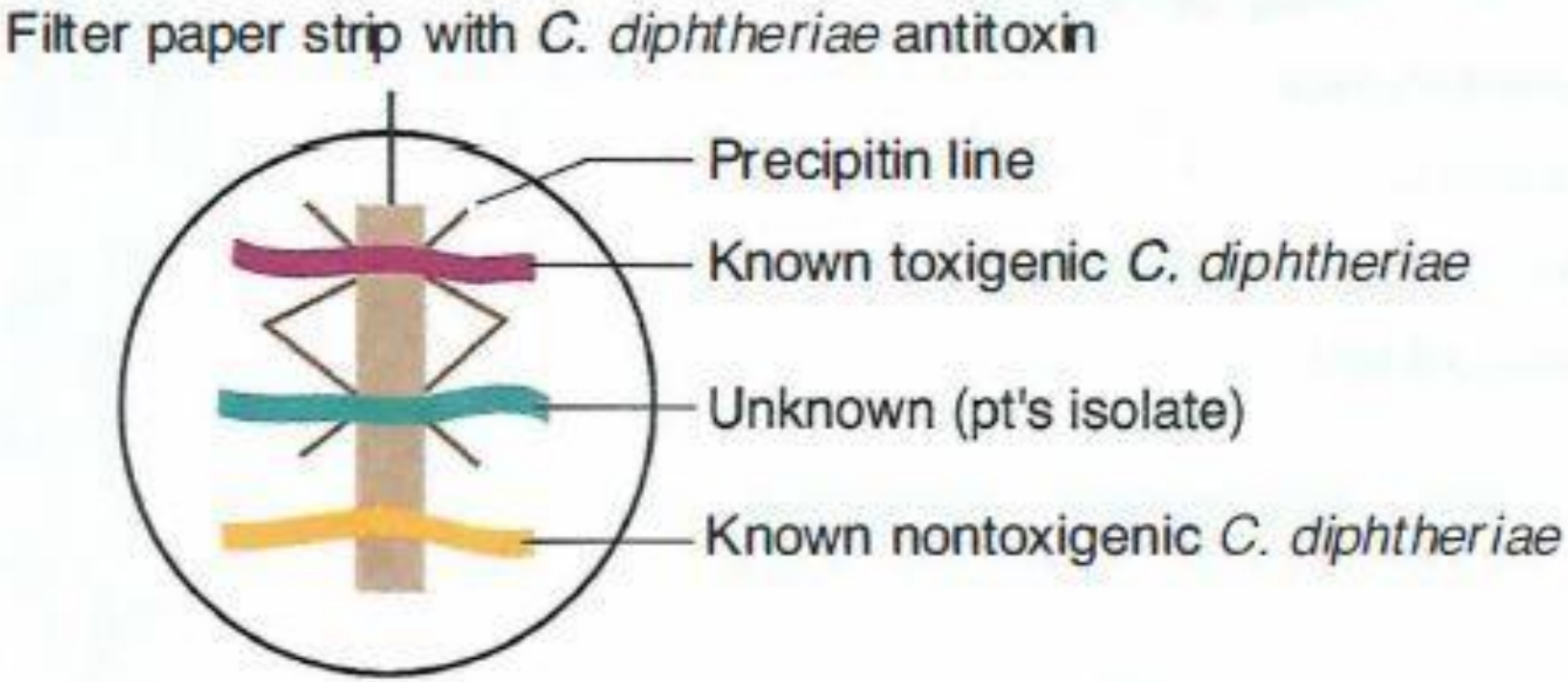
- All the enzymic activity of the toxin resides in fragment A.
- This fragment alone unable to enter the cell with out the hydrophobic fragment B, which provides a mechanism for the transport and attachment of fragment A to sensitive sites on the cell membrane. fragment B has no independent activity
- 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.

In vitro gel precipitation test : (Elek's test)

- Elek's medium contained horse serum
- Place 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 ..

Elek's test


Filter paper strip with C. diphtheriae antitoxin
Precipitin line
Known toxigenic C. diphtheriae
Unknown (pt's isolate)
Known nontoxigenic C. diphtheriae

Pathogenicity

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

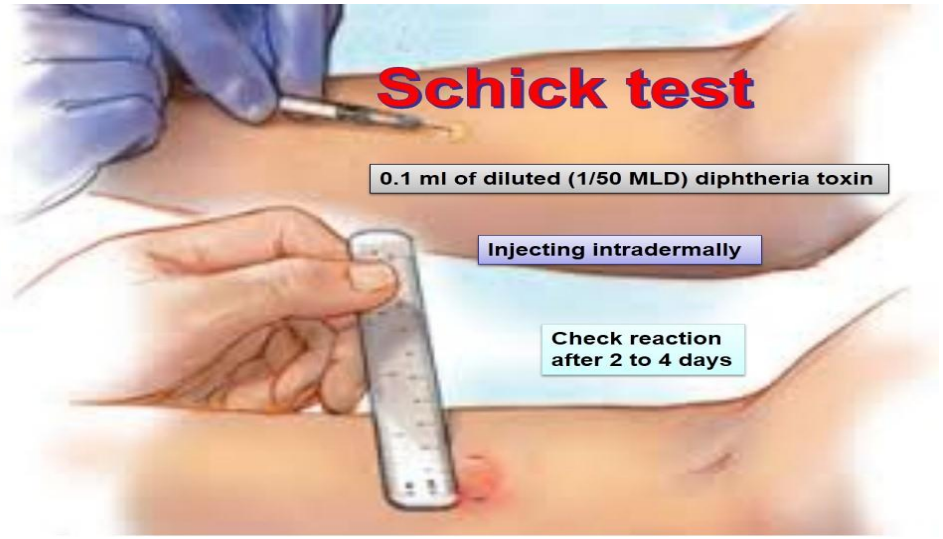
- but it soon become thick and tough forming a dull white layer or pseudomembrane covering the area.
- the initial lesion may cover tonsil and then extend further and patient may show manifestations of toxemia, with relatively low fever with temperature 38C to 39C laryngeal involvement it may result in mechanical stoppage of the air passage and death of the patient.

Pseudomembrane of diphtheria

Schick test

- In 1913 schick described test based on the fact that when a minute amount of diphtheria toxin is introduced intradermally
- It excretes 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**



Treatment

- Treatment with antitoxin is mandatory when there is strong clinical suspicion of diphtheria.
- Antimicrobial drugs (penicillin, erythromycin) inhibit the growth of diphtheria bacilli.

Prevention

- Artificial immunization, diphtheria was mainly a disease of small children.

Active immunization in childhood with diphtheria toxoid yields antitoxin levels that are generally adequate until adulthood.