True bacteria – Rods - Gram positive rods

Non-spore forming bacteria

Corynebacterium, Listeria, Propionibacterium

Corynebacterium

The genus *Corynebacterium* includes *C. diphtheriae*, the cause of the diphtheria, and *Diphtheriodes*, as a large genus of the normal flora (the skin, the conjunctival sac, the mouth, the vagina).

Corynebacterium diphtheriae

Diphtheria, caused by *C. diphtheriae* is an acute respiratory or cutaneous disease and may be a life-threatening illness. The vaccination protocols and widespread immunization beginning in early childhood has made the disease rare in developed countries while is a serious disease in those countries where the population has not been immunized.

Epidemiology

C. diphtheriae is found in the throat and nasopharynx of carriers and in patients with diphtheria. The disease is a local infection, so the organism is primarily spread by:

1- **respiratory droplets** by carriers.

2-**direct contact** with an infected individual

3-**contaminated waste**.

Pathogenesis

Diphtheria is caused by the effects of a exotoxin that inhibits eukaryotic protein synthesis. The toxin is composed of two

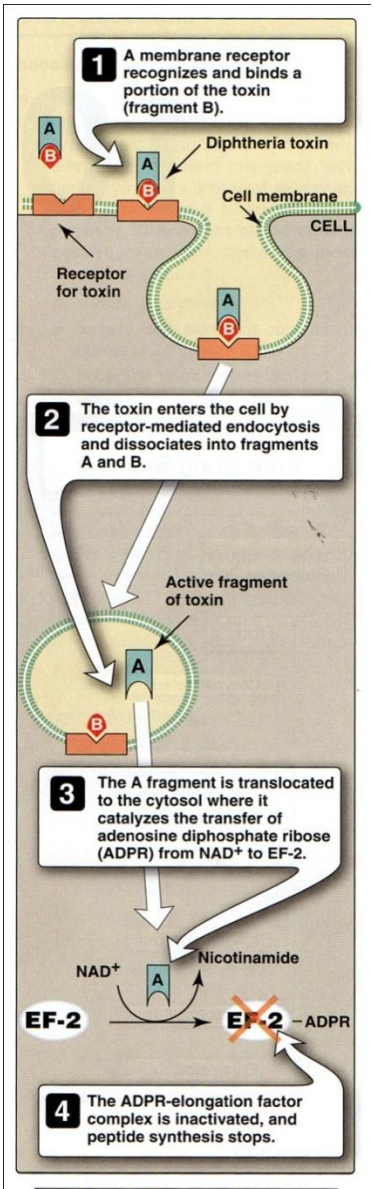

Figure 1
Action of diphtheria toxin.

fragments A and B(Figure 1). Fragment B binds to cell membranes (by the receptor in cell membrane) and then mediates the delivery of fragment A to it's target. Inside the cell, fragment A separates from fragment B, and catalyzes the transfer of adenosine diphosphate ribose (ADPR) from nicotine adenine dinucleotid (NAD^+) to polypeptide chain elongation factor EF-2 ,then,the complex(ADPR+EP-2)is inactivated and peptide synthesis stops.

Clinical Significance

Infection has two forms respiratory and cutaneous or in an asymptomatic carrier

1-Respiratory diphtheria

Diphtheria consist of (1) local infection of the throat, produces a thick, grayish, adherent exudate (pseudomembrane) that is composed of cell debris from the mucosa and inflammatory products (Figure 2), coats the throat and may extend into the nasal passages or down ward in the respiratory tract, leading to suffocation.

(2) generalized symptoms occur as the disease progresses (marked swelling of the lymph nodes in the neck), involve the heart and peripheral nerves, lead to congestive heart failure and permanent heart damage and neuritis of cranial nerves and paralysis of muscle groups (such as those control movement of the palate or the eye) are seen late in the disease.

2- Cutaneous diphtheria

A puncture wound or cut in the skin can result in introduction of *C. diphtheriae* into the subcutaneous tissue, leading to a chronic, non healing ulcer with a gray membrane.

Immunity

Diphtheria toxin is antigenic and stimulates the production of Abs that neutralize the toxin's activity. Formalin treatment of the toxin produces a **toxoid** that remains the antigenicity but not the toxicity of the molecule, which used for immunization against the disease.

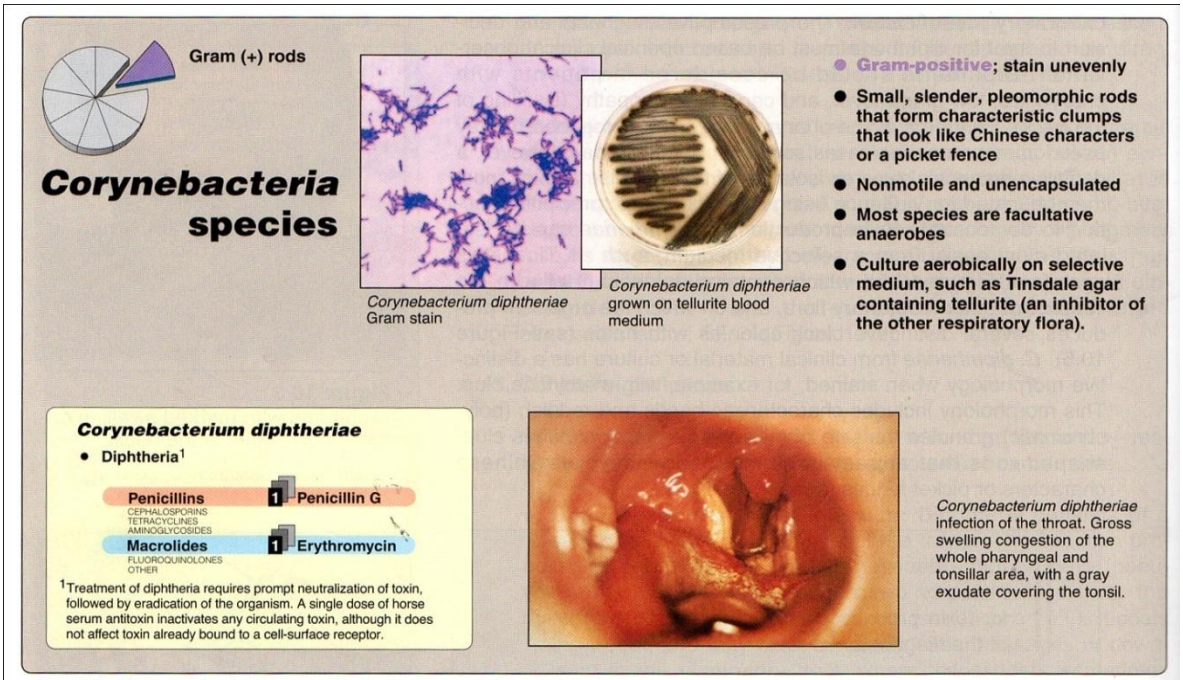

Figure
Summary of *Corynebacterium diphtheriae* disease.

Diagnostic laboratory tests

1- Clinical observation → Diphtheria should be considered in patients with = pharyngitis, low –grade fever,cervical adenopathy (swelling of the neck),erythema of the pharynx and adherent gray pseudomembranes.

2- Microscopic examination: G+ rods ,small, slender, pleomorphic,that tend to stain unevenly,form clumps that look like Chinese characters , nonmotile, noncapsulated and do not form spores. Albert's stain is important to diagnostic *C.* because its contain volutin granules (*C.* contains accumulation of phosphate granules, metachromatic granules) that stain deeply give rod a beaded appearance beside the bacilli looks like bipolar under the microscope(see figure 3) and arranged in pairs or groups and from various angles with each other like L, V, X, Y, Z thus called Chinese letter arrangement.

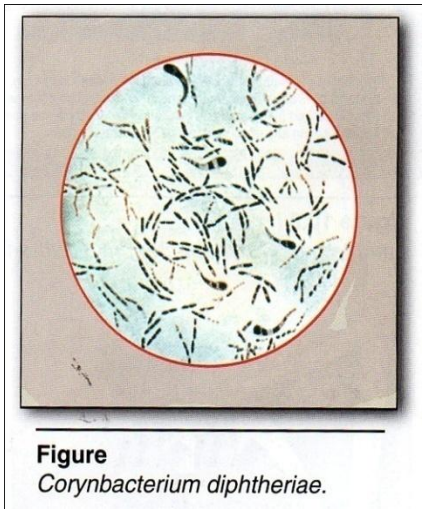

Figure
Corynbacterium diphtheriae.

3-Macroscopic examination: Culture = Most spp. are facultative anaerobic and those found associated with humans grow aerobically on standard laboratory media such as blood agar. *C. diphtheriae* can be isolated easily from a selective medium such as Tinsdale's agar (Figure 2) which contains potassium tellurite, an inhibitor of other respiratory flora, and on which *C.* produces black colonies with halos (to reduction of tellurite salt). On rich media (blood, serum or egg) colonies are small, granular, moist, glistening irregular edges.

Biochemical reaction = Catalas +, oxidase - , gelatin- , Urase - , phosphatase -, ferment glucose and maltose with acid production only.

Fermentation of starch, glycogen and dextrin, is useful to differentiate between the 3 types of *C. diphtheriae* (on selective media containing tellurite, 3 types of *C. diphtheriae* : **Gravis**, **Mitis**, **Intermedius**, all of them capable of producing same toxin and clinical disease). Gravis ferments starch, glycogen and dextrin, while the two other types don't ferment starch and glycogen,ferment only dextrin.

Serologic test : by precipitin reaction to demonstrate toxin production.

Treatment

Requires (1) neutralization of toxin: by A single dose of horse serum antitoxin inactivates any circulating toxin, not bound toxin to a cell-surface receptor.

(2) eradication of the organism: by several antibiotics, such as erythromycin or penicillin (Figure 2), which slows the spread of infection and, by killing the organism, prevents further toxin production.

Prevention

By vaccine contains diphtheria toxoid as a part of the DTaP vaccine (**D**iphtheria + **T**etanus + a **P**ertussis vaccine)which neutralize unbound toxin preventing the disease from progressing. The initial series of injections should be started in children (2 years). Booster injections of diphtheria toxoid (with tetanus toxoid) should be given at 10 year intervals throughout life. The control of an epidemic outbreak of diphtheria involves rigorous immunization and a search for healthy carriers among patient contacts.

Diphtheroids

Several other *Corynebacterium spp.*, that morphologically resemble *to C. diphtheriae* , **are common commensals of the nose, throat, nasopharynx, skin, urinary tract and conjunctiva,** and are generally **unable to produce exotoxin, but a few cause disease in immunosuppressed individuals.**

Listeria

Listeria species **are intracellular parasites that may be seen within host cells in tissue samples** (Figure 1) . *Listeria monocytogenes* is the only species that infects humans, other species are widespread among animals.

Figure 1
Summary of *Listeria* species.

Epidemiology

Listeria infections are usually food borne, 2 - 3 % of processed dairy products (ice cream and cheese), 20 - 30 % of ground meats and poultry (growth at 4°C in food).

1 – 15 % of healthy humans are asymptomatic intestinal carriers of the organism. *Listeria* infections are most common in pregnant women + fetuses or newborns + immunocompromised individuals, such as the elderly or patients receiving corticosteroids.

Antigenic classification

Three serotypes are present isolates from humans , Ia , Ib , Irb .

Irb causes an epidemic associated with cheese made from anpasteurized milk,cause meningitis between birth 3 week of life with high mortality rate .

Pathogenesis

It's a facultative intracellular parasite, attaches to and enters a variety of mammalian cells, by phagocytosis(macrophage)and incorporated into a phagolysosome (see figure) .

It escapes from the phagolysosome by elaborating a toxin called **Listeriolysin O** as virulent factors

L. monocytogenes grows in the cytosol, and assembles an actin filament tail that pushes the bacterium to the surface of the macrophage .

A pseudopod extension forms , facilitating transfer of the *Listeria* into a neighboring phagocyte. *Listeria* produced **phospholipases** mediate the passage of the organism directly to a neighboring cell, allowing avoidance of cells of the immune system.

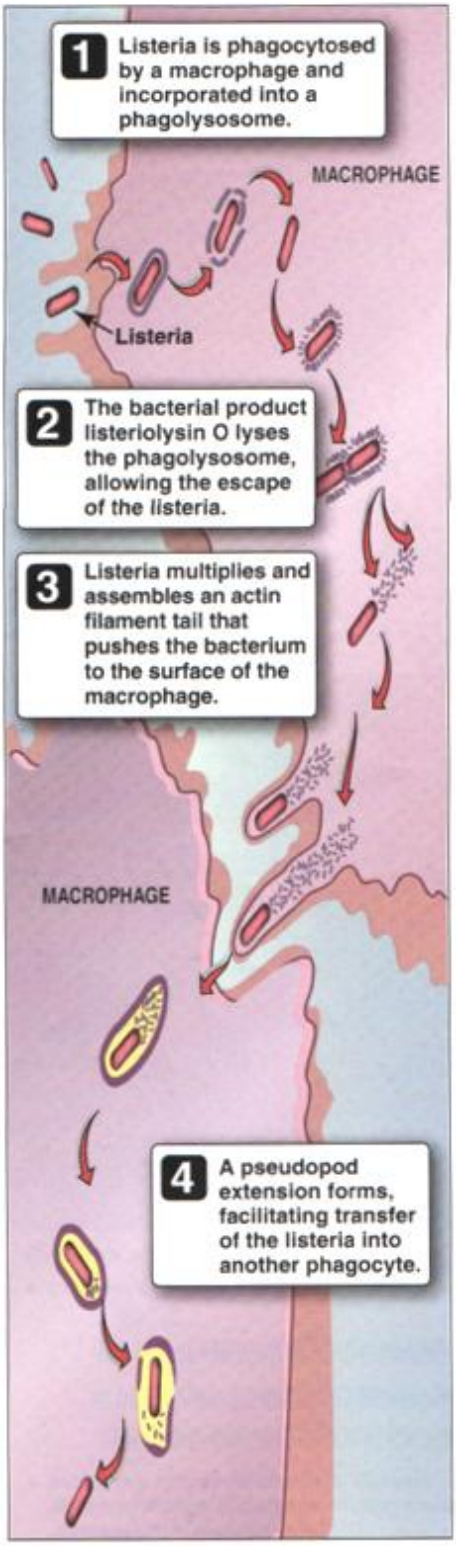

Figure
Life cycle of *Listeria monocytogenes* in host macrophages.

Clinical significance

Septicemia and meningitis are the most commonly reported forms of *L. monocytogenes* infection (listeriosis).

Pregnant women, usually in the third trimester, may have a milder illness as well as in asymptomatic vaginal colonizaition, the organism can be transmitted to a newborn (cause of newborn meningitis) or to the fetus and initiate abortion. Immunocompromised individuals are susceptible to serious generalized infections, as meningocephalitis and bacteremia .

Diagnostic laboratory tests

Specimens = blood, cerebrospinal fluid, and other clinical specimens by standard bacteriologic procedures.

Microscopic examination = G+ rods, sometimes they occur as diplobacilli or in short chains, do not form spores and they are intracellular parasites that may be seen within host cells in tissue samples. Its **motile with tumbling motility by light microscopy in liquid medium after growth at 25°C , distinguish it from *Corynebacterium* (nonmotile) which may be confused morphologically with *Listeria*.**

Macroscopic examination = It's facultative anaerobes on a variety of enriched media. On blood agar, *L. monocytogenes* produces a small colony with β- hemolysis(figure 1).

Biochemical reaction = **catalase + distinguish it from *Streptococci* (catalase -) which may be confused morphologically with *Listeria*.**

Treatment

Ampicillin and trimethoprim /sulfamethoxazole are used (Figure 1). Ampicillin and gentamicin also used after therapy.

Prevention

By proper food preparation and handling.

Propionibacterium

Propionibacterium acnes is classified as a *Corynebacterium* anaerobic diphtherods, form part of the normal flora of the skin.They cause **acne** by produce **lipase** which split off free fatty acids from skin ,then produce tissue inflammation which contribute to acnes . Sometimes associated with endocarditis or infections of plastic implants. It's produce propione acid from carbohydrate fermentation . Its G+ rods , non spore forming , anaerobic microaerophilic rods of diphtheroid like morphology.