Aerobic Non–Spore-Forming Gram-Positive Bacilli:

Ex:

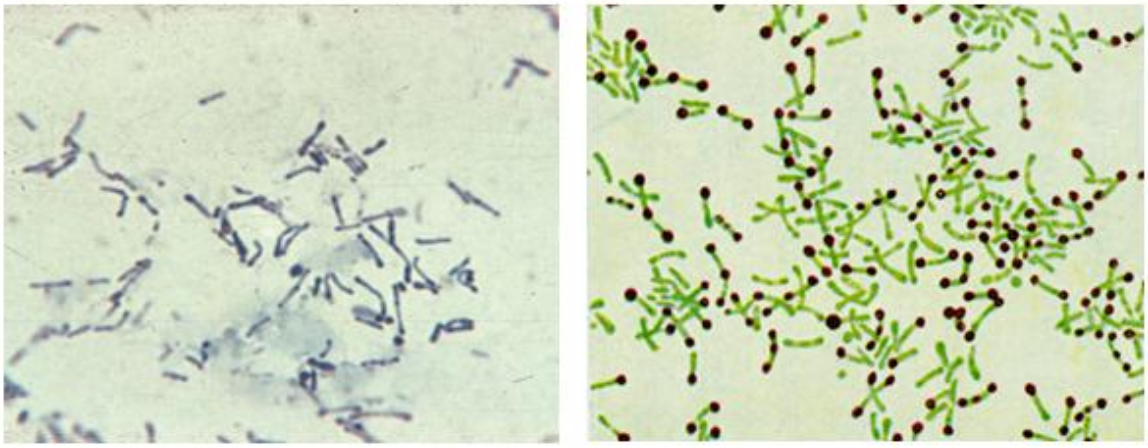
1- ***Corynebacterium* (*Corynebacterium diphtheria*)**
2- ***Listeria* (*Listeria monocytogenes*)**
3- ***Propionibacterium* (*Propionibacterium acnes*)**

The non-spore-forming gram-positive bacilli are a diverse group of bacteria. Many members of the genus *Corynebacterium* and their anaerobic equivalents, *Propionibacterium* species, are members of the normal flora of skin and mucous membranes of humans. Other corynebacteria are found in animals and plants. *Corynebacterium diphtheriae* is the most important member of the group, as it can produce a powerful exotoxin that causes diphtheria in humans. *Listeria monocytogenes* is primarily found in animals and occasionally cause severe disease in humans.

Corynebacterium diphtheria

- Morphology

- They possess irregular swellings at one end that give them the "**club-shaped**" appearance. Irregularly distributed within the rod (often near the poles) are granules staining deeply with aniline dyes (metachromatic granules) that give the rod a beaded appearance. "Chinese letters" , Albert's stain – green and bluish black ,non motile ,non capsulated, non-spore forming.
- On blood agar, the *C.diphtheriae* colonies are small, granular, and gray with irregular edges and may have small zones of hemolysis. On agar containing potassium tellurite, the colonies are brown to black with a brown-black halo because the tellurite is reduced intracellularly. Four biotypes of *C diphtheriae have been widely recognized: gravis, mitis, intermedius,* and *belfanti*.

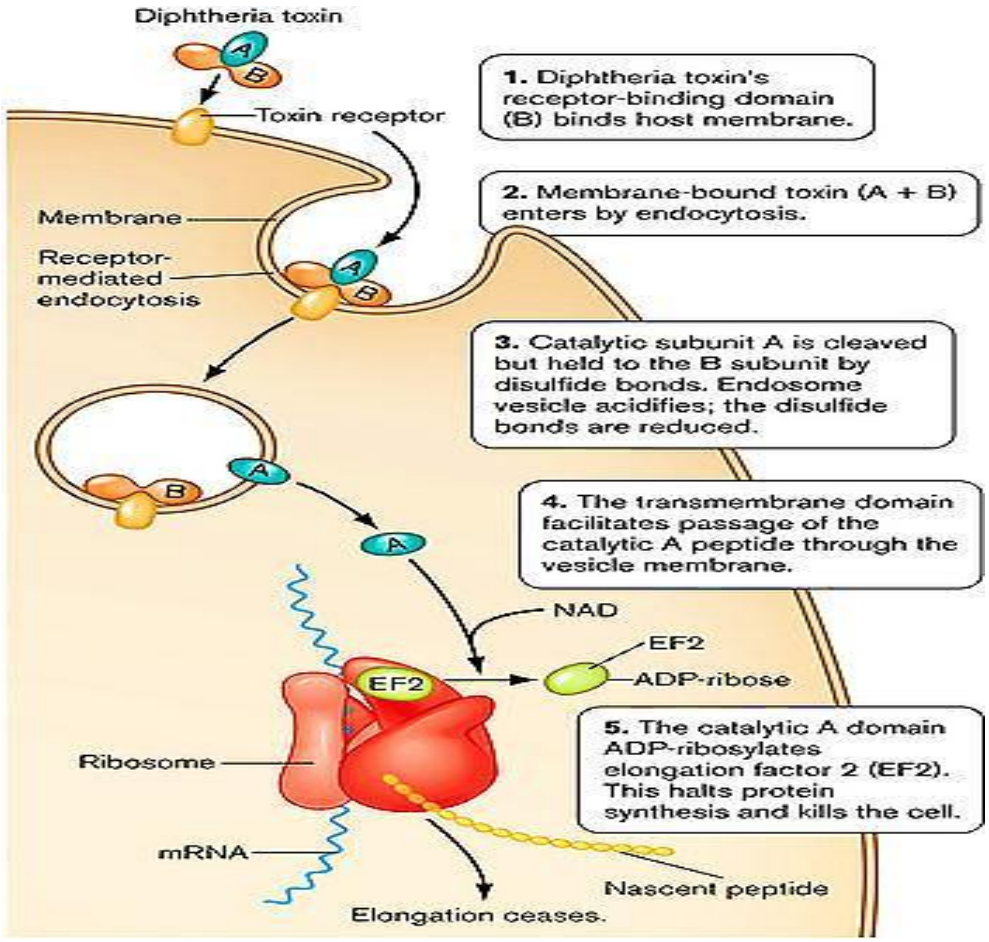


Corynebacterium diphtheriae stained with methylene blue.

***C.diphtheriae* on blood agar**

- Pathogenesis

- The principal human pathogen of the genus *Corynebacterium* is *C.diphtheriae*, the causative agent of **respiratory** or **cutaneous diphtheria**. In nature, *C diphtheriae* occurs in the respiratory tract, in wounds, or on the skin of infected persons . It is spread by droplets or by contact to susceptible individuals; the bacilli then grow on mucous membranes or in skin abrasions, and those that are toxigenic start producing toxin.
- Diphtheria toxin is absorbed into the mucous membranes and causes destruction of epithelium and a superficial inflammatory response.



Mechanism of diphtheria toxin action

The necrotic epithelium becomes embedded in exuding fibrin and red and white cells, so that a grayish "pseudomembrane" is formed commonly over the tonsils, pharynx, or larynx. Any attempt to remove the pseudomembrane exposes and tears the capillaries and thus results in bleeding. The regional lymph nodes in the neck enlarge, and there may be marked edema of the entire neck, with distortion of the airway, often referred to as "**bull neck**"

(Bull neck)

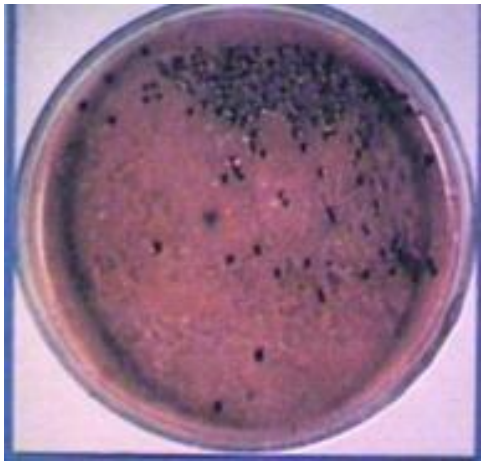
- **Clinical Findings**

When diphtheritic inflammation begins in the respiratory tract, sore throat and low-grade fever usually develop. Prostration and dyspnea soon follow because of the obstruction caused by the membrane. This obstruction may even cause suffocation if not promptly relieved by intubation or tracheostomy. Irregularities of cardiac rhythm indicate damage to the heart. Later there may be difficulties with vision, speech, swallowing, or movement of the arms or legs. All of these manifestations tend to subside spontaneously.

-Specialized media:

A-Tellurite:

Black colonies not diagnostically significant, tellurite inhibits many organisms but not *C. diphtheria*.



- Selective & differential medium
- Corynebacteria are resistant to tellurite
  - Reduced to tellurium
- Forms deposit in colonies
  - Colonies appear dark

B-Loeffler

- On **Loeffler serum medium**, corynebacteria grow much more readily than other respiratory organisms, and the morphology of organisms is typical in smears made from these colonies.

Pseudomembrane

Contains:

- bacteria - fibrin
- dead cells - lymphocytes
- plasma cells

Laboratory diagnosis:

- Sample collection: Throat swab or swab from membrane
- Microscopy: Gram stain and Alberts stain
- **Culture?**
- Biochemicals tests.
- Immunologic assays to determine the diphtheria toxin

Treatment

The treatment of diphtheria rests largely on rapid suppression of toxin-producing bacteria by antimicrobial drugs and the early administration of specific antitoxin against the toxin formed by the organisms at their site of entry and multiplication.

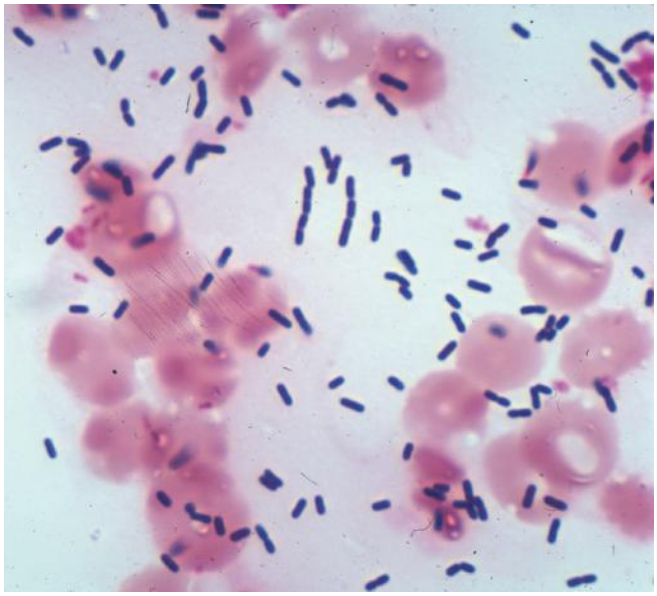
Antimicrobial drugs (**Penicillin, Erythromycin**) inhibit the growth of diphtheria bacilli and arrest toxin production.

Listeria monocytogenes (Cause **Listeriosis**)

There are several species in the genus *Listeria*. Of these, *L monocytogenes is* important as a cause of a wide spectrum of disease in animals and humans. *L monocytogenes* is capable of growing and surviving over a wide range of environmental conditions. It can survive at refrigerator temperatures (4°C), under conditions of low pH and high salt conditions. Therefore, it is able to overcome food preservation and safety barriers, making it an important **foodborne pathogen**.

-Morphology and Identification:

L.monocytogenes is a short, gram-positive, non–spore-forming rod. It is **catalase** positive and has a tumbling end-over-end ,motility at 22–28°C but not at 37°C; the motility test rapidly differentiates *Listeria* from *diphtheroids* .



Gram stain of the gram-positive bacillus *Listeria monocytogenes*

Culture and Growth Characteristics

Listeria grows well on media such as 5% sheep blood agar on which it exhibits the characteristic small zone of hemolysis around and under colonies. The organism is a **facultative anaerobe** and is **catalase positive** and **motile.** Listeria produces **acid** but not gas from utilization of a variety of carbohydrates. The motility at room temperature and **hemolysin** production are primary findings that help differentiate *Listeria* from coryneform bacteria.

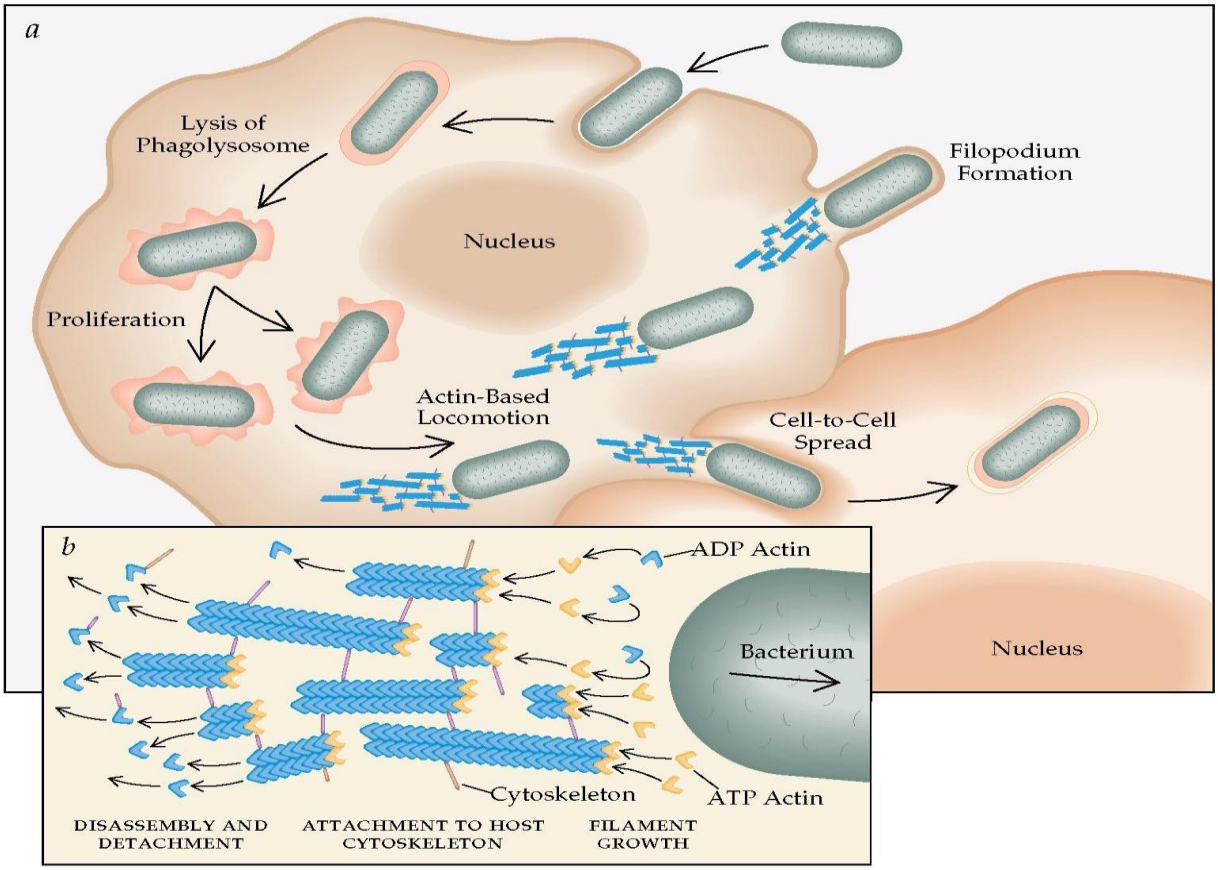
Antigenic Classification:

Serologic classification is done only in reference laboratories . There are 13 known serovars based on O (somatic) and H (flagellar) antigens.

Pathogenesis:

L monocytogenes enters the body through the gastrointestinal tract after ingestion of contaminated foods such as cheese or vegetables. The organism has several adhesin proteins **(Ami, Fbp A, and flagellin proteins)** that facilitate bacterial binding to the host cells and that contribute to virulence. It has cell wall surface proteins called internalins A and B that interact with **E-cadherin**, a receptor on epithelial cells, promoting phagocytosis into the epithelial cells.

After phagocytosis, the bacterium is enclosed in a phagolysosome, where the low pH activates the bacterium to produce **listeriolysin O**. This enzyme lyses the membrane of the phagolysosome and allows the *Listeriae* to escape into the cytoplasm of the epithelial cell. The organisms proliferate, and Act A, another listerial surface protein, induces host cell actin polymerization, which propels them to the cell membrane. Pushing against the host cell membrane, they cause formation of elongated protrusions called **filopods**. These filopods are ingested by adjacent epithelial cells, macrophages, and hepatocytes, the *Listeriae* are released, and the cycle begins again.



Clinical Findings

There are two forms of perinatal human listeriosis. Early onset syndrome (granulomatosis infantiseptica) is the result of infection in utero and is a disseminated form of the disease characterized by neonatal sepsis, pustular lesions, and granulomas containing *L monocytogenes in multiple organs. Death* may occur before or after delivery.

The late-onset syndrome causes the development of meningitis between birth and the third week of life and has a significant mortality rate.

Adults can develop *Listeria meningoencephalitis*, *bacteremia*, and (rarely) focal infections. Meningoencephalitis and bacteremia occur most commonly in immunosuppressed patients, in whom *Listeria* is one of the more common causes of meningitis.

Propionibacterium

- *Propionibacterium* species are members of the normal microbiota of the skin, oral cavity, large intestine, conjunctiva, and external ear canal. Is gram positive, anaerobic. Their metabolic products include propionic acid, from which the genus name derives. On Gram stain, they are highly pleomorphic, showing curved, clubbed, or pointed ends; long forms with beaded uneven staining; and occasionally coccoid or spherical forms. *Propionibacterium acnes*, often considered an opportunistic pathogen, causes the disease acne vulgaris and is associated with a variety of inflammatory conditions. It causes acne by producing lipases that split free fatty acids off from skin lipids. These fatty acids can produce tissue inflammation that contributes to acne formation.

- In addition, *P.acnes* is frequently a cause of postsurgical wound infections, particularly those that involve insertion of devices, such as prosthetic joint infections, particularly of the shoulder, central nervous system shunt infections, osteomyelitis, endocarditis, and endophthalmitis. Because it is part of the normal skin microbiota, *P.acnes* sometimes contaminates blood or cerebrospinal fluid cultures that are obtained by penetrating the skin. It is therefore important (but often difficult) to differentiate a contaminated culture from one that is positive and indicates infection.