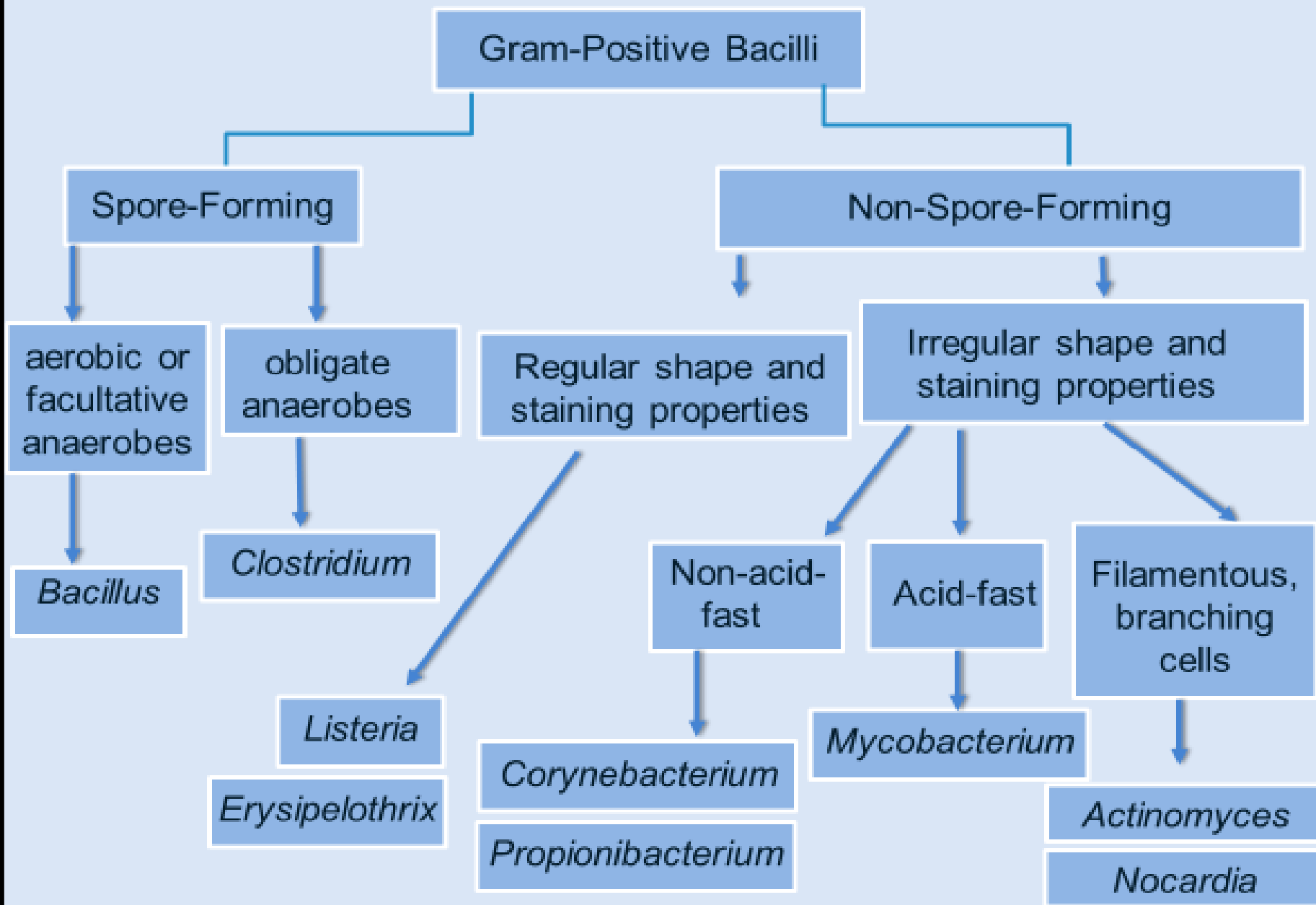




PHARMACEUTICAL MICROBIOLOGY

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OBJECTIVES

Non-Spore-Forming Gram-Positive Bacilli:

- *Listeria monocytogenes*
- *Erysipelothrix rhusiopathiae*
- *Corynebacterium diphtheriae*



Non-Spore-Forming Gram-Positive Bacilli

- Gram positive rods that are **uniform in shape** include **Listeria and Erysipelothrix**.
- The coryneform rods (which include the genus **Corynebacterium**) comprise a large group of **irregularly shaped rods**.

Non-Spore-Forming Gram-Positive Bacilli

- The other rod-shaped bacteria is characterized by the presence of **long-chain mycolic acids** in their cell walls.
- This cell wall component **makes it difficult to stain** the bacteria with the Gram stain; thus the **acid-fast stain** was developed.
- Bacteria that are partially or completely acid-fast include **Nocardia, Rhodococcus** and **Mycobacterium**.

Listeria monocytogenes

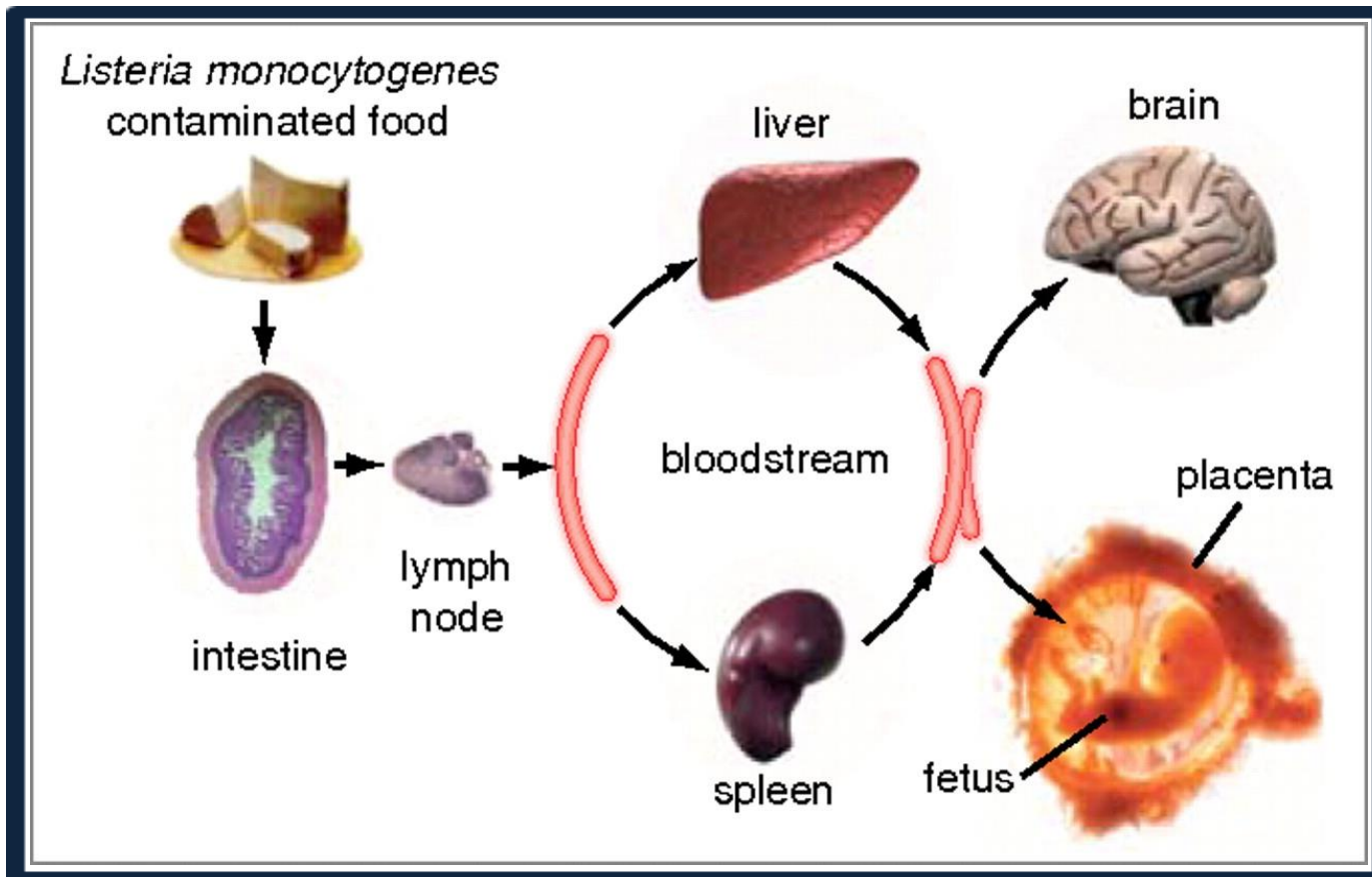
- *Listeria monocytogenes* is a Gram-positive, **non-spore-forming, motile, facultatively anaerobic**, rod-shaped bacterium.
- It is **catalase-positive** and **oxidase-negative**, and expresses a **beta hemolysin**, which causes destruction of red blood cells.
- *Listeria* grows well on media such as 5% sheep blood agar on which it exhibits the characteristic **small zone of hemolysis around and under colonies**.

Listeria monocytogenes

- This bacterium exhibits characteristic **tumbling motility** when viewed with light microscopy.
- Although *L. monocytogenes* is actively motile by means of **peritrichous flagella** at room temperature (20–25 °C), **the organism does not synthesize flagella at body temperatures (37 °C)**. They are non-motile at 37 °C.
- **The motility at room temperature and hemolysin production** are primary findings that help **differentiate Listeria from Coryneform**.

Listeria monocytogenes

- *Listeria monocytogenes* is a facultative intracellular pathogen that can grow in macrophages, epithelial cells.



Listeria monocytogenes

- Entry into non-phagocytic cells is mediated by a family of six or more **leucine-rich proteins, internalins (e.g. InIA, InIB, InIC)**, which interact with glycoprotein receptors on the surface of host cells.
- After penetration into the cells, the acid pH of the **phagolysosome** that surrounds the bacteria activates a **bacterial exotoxin (listeriolysin O)** and two different **phospholipase C enzymes**, leading to release of the bacteria into the cell cytosol.

Listeria monocytogenes

- The bacteria proceed to replicate and then move to the cell membrane. This movement is mediated by a bacterial protein, **ActA**, localized on the cell surface at one end of a bacterium, that coordinates assembly of **actin**.
- Entry into macrophages after passage through the intestinal lining carries the bacteria to the **liver and spleen**, leading to disseminated disease.

Listeria monocytogenes

- Humoral immunity is relatively unimportant for management of infections with *L. monocytogenes*. These bacteria can replicate in macrophages and move within cells, thus avoiding antibody-mediated clearance.
- *L. monocytogenes* is isolated from a variety of environmental sources and from the feces of mammals, birds, fish, insects and other animals. The primary sources of this organism are believed to be soil and decaying vegetable matter. **People usually become ill with listeriosis after eating contaminated foods.**

Listeria monocytogenes

- Foodborne infection in humans occurs through the **consumption of contaminated foods**, particularly **unpasteurized milk, soft cheeses, vegetables and prepared meat products**.
- Unlike most foodborne pathogens, *Listeria* multiplies readily in refrigerated foods that have been contaminated. **It 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.**

Listeria monocytogenes

- **Listeriosis** is a serious infection caused by this bacteria
- Infection with *L. monocytogenes* may be asymptomatic, or it may result in a spectrum of clinical presentations including **acute non-febrile or febrile gastroenteritis, sepsis, or meningitis.**
- The disease primarily affects pregnant women, newborns, older adults, and people with weakened immune systems.

Listeria monocytogenes

- **Listeriosis** during pregnancy is typically a relatively mild “**flu-like**” illness. Some pregnant women with *Listeria* infection have no symptoms. Although severe disease in the mother is rare, infection during pregnancy can result in **miscarriages, stillbirths, preterm labor, and sepsis or meningitis in the neonate.**
- Some neonates with listeriosis develop **granulomatosis infantiseptica**, a severe disorder involving the internal organs and skin.

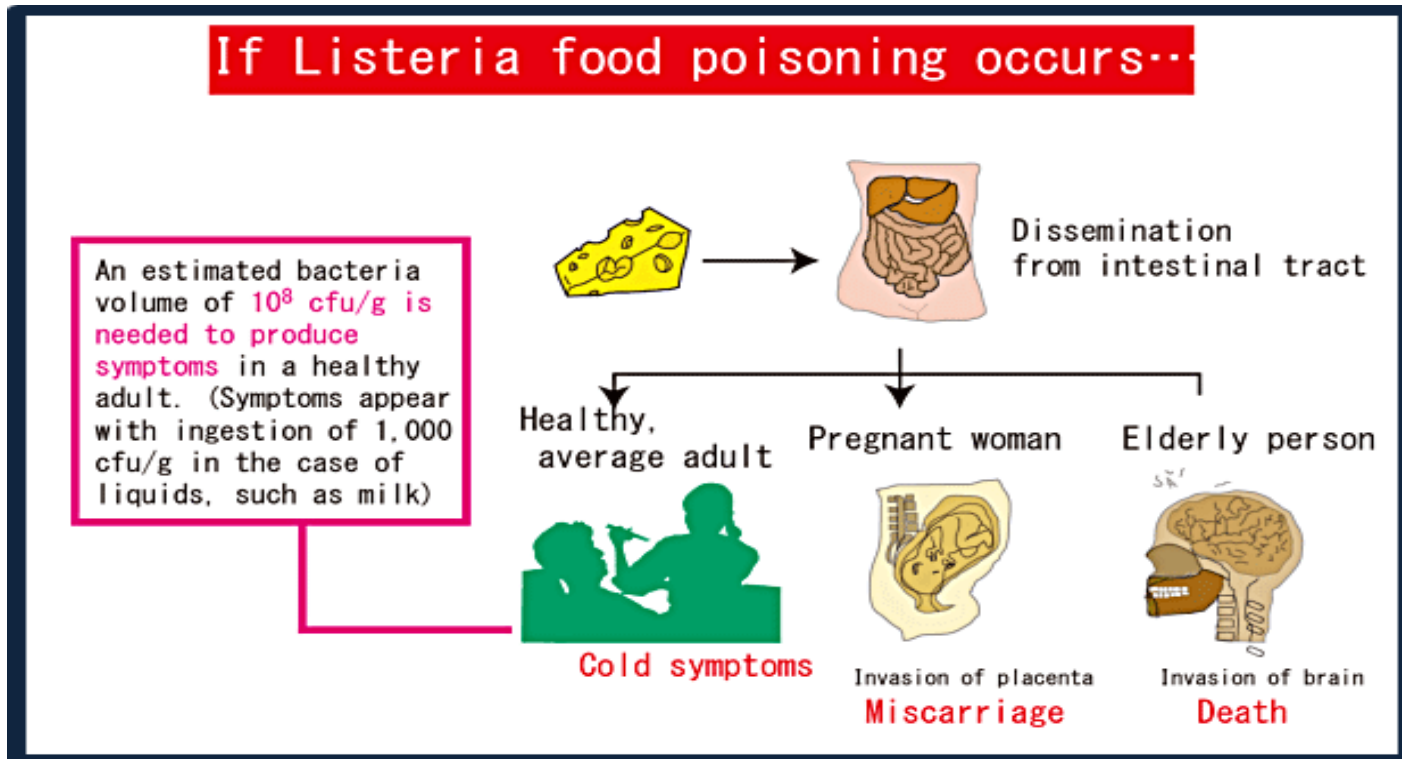
Listeria monocytogenes

Neonatal listeriosis is classified as **early (within 6 days of birth)** or **late onset (7–28 days after birth)**.

- **Early-onset neonatal listeriosis** is usually acquired **through transplacental transmission**.
- The sources of **late-onset listeriosis** are less clear; they may involve **exposure during delivery** or **nosocomial exposure**.

Listeria monocytogenes

In older adults and people with weakened immune systems, severe infections of the bloodstream (causing **sepsis**) or brain (causing **meningitis** or **encephalitis**) can be seen.



Listeria monocytogenes

Pregnant women, fetuses, and newborn infants



Listeria can pass from pregnant women to their fetuses and newborns. It can cause miscarriages, stillbirths, and newborn deaths.



Chancy cheese

LISTERIA OUTBREAK: Queso fresco (a type of soft cheese) sickened 142 people, killed 10 newborns and 18 adults, and caused 20 miscarriages.

People with weakened immune systems



Listeria can spread through the bloodstream to cause meningitis, and often kills. The weaker your immune system, the greater the risk.



Contaminated celery

LISTERIA OUTBREAK: Pre-cut celery in chicken salad served at hospitals sickened 10 people who had other serious health problems. Five of them died as a result.

Adults 65 or older



Listeria can spread through the bloodstream to cause meningitis, and often kills. The older you are, the greater the risk.



Tainted cantaloupes

LISTERIA OUTBREAK: Contaminated whole cantaloupes sickened 147 people in 28 states and caused one of the deadliest foodborne outbreaks in the US. There were 33 deaths, mostly in adults over 65, reported during the outbreak.

SOURCE: CDC, 2013

What foods are risky?

When it comes to *Listeria*, some foods are more risky than others. Meet some of the other foods where *Listeria* is known to hide.



Raw Sprouts



Raw Milk
(unpasteurized)



Soft Cheeses



Deli Meats and Hot Dogs
(cold, not heated)



Smoked Seafood

Listeria monocytogenes

- *L. monocytogenes* is not fastidious and may easily be cultured from blood, spinal fluid, or the placenta using standard blood culture techniques and laboratory identification protocols.
- Polymerase chain reaction (PCR) is sensitive and specific for the diagnosis of meningitis due to *L. monocytogenes*.

Listeria monocytogenes

- Currently, **penicillin or ampicillin, either alone or with gentamicin**, is the **treatment of choice** for infections with *L. monocytogenes*.
- **Listeriae are naturally resistant to cephalosporins.**
- Erythromycin can be used in patients allergic to penicillin, but **resistance to trimethoprim and the tetracyclines** has been observed.

Listeria monocytogenes

- **Resistance to aminoglycosides** has also been reported.
- Genes for **resistance to tetracyclines and aminoglycosides** have been found on **conjugative plasmids and transposons** that **originated in enterococci**.

Listeria monocytogenes

These are the recommendations for preventing listeriosis:

- Do not drink raw (unpasteurized) milk or eat foods that contain unpasteurized milk.
- Wash raw vegetables thoroughly before eating.
- Keep the refrigerator temperature at 4 °C or lower; the freezer at -18 °C or lower.
- Eat precooked, perishable, or ready-to-eat food as soon as possible.

Listeria monocytogenes

These are the recommendations for preventing listeriosis:

- Keep raw meat, fish, and poultry separate from other food that will not be cooked and from cooked foods and ready-to-eat foods.
- Wash hands, knives, and cutting boards after handling uncooked food.
- Thoroughly cook raw food from animal sources to a safe internal temperature: ground beef 71 °C, chicken 77 °C, turkey 82°C.

Erysipelothrix rhusiopathiae

- *Erysipelothrix rhusiopathiae* is a **Gram-positive, non-spore forming, microaerophilic, facultatively anaerobic rod** that is distributed worldwide in wild and domestic animals.
- The rods are slender and sometimes pleomorphic, with a tendency to form filaments as long as 60 μm (**hairlike**).
- **Small, grayish, α -hemolytic colonies** are observed **after 2 to 3 days of incubation**.

Erysipelothrix rhusiopathiae

The following two primary forms of human infection with *E. rhusiopathiae* have been described:

- **a localized skin infection (erysipeloid)**
- **a septicemic form**

Erysipeloid is an acute bacterial infection of traumatized skin and other organs. Direct contact between meat infected with *E. rhusiopathiae* and traumatized human skin results in erysipeloid.

Erysipelothrix rhusiopathiae

- In animals, the organism causes swine erysipelas and several other diseases in poultry and sheep.
- Humans acquire erysipeloid after direct contact with infected animals.
- **Erysipeloid** is more common among farmers, butchers, cooks, homemakers, and anglers.

Erysipelothrix rhusiopathiae

- Penicillins and cephalosporins are the first-line choice for treatment. Tetracyclines, quinolones, clindamycin, erythromycin, imipenem, and piperacillin can be used for treatment.
- It is resistant to vancomycin, chloramphenicol, daptomycin, gentamicin, netilmicin, polymyxin B, streptomycin, teicoplanin, tetracycline, and trimethoprim/sulfamethoxazole.

Corynebacterium

- The genus *Corynebacterium* is a large, heterogeneous collection of species that have a **cell wall with arabinose, galactose, meso-diaminopimelic acid (meso-DAP)** and (in most species) short-chain **mycolic acids**.
- Gram stains of these bacteria reveal clumps and short chains (**V and Y configurations**) of **irregularly shaped rods**.
- Metachromatic granules within the cells may be seen with special stains.

Corynebacterium

- Corynebacteria are **aerobic or facultatively anaerobic, non-motile** and **catalase positive**.
- Most (but not all) species ferment carbohydrates, producing lactic acid as a by-product.
- Many species grow well on common laboratory media; however, some species require supplementation of media with lipids for good growth (lipophilic strains).

Corynebacterium

- Corynebacteria are ubiquitous in plants and animals, and they normally colonize the skin, upper respiratory tract, gastrointestinal tract and urogenital tract in humans.
- Although all species of Corynebacteria can function as opportunistic pathogens, a few are more commonly associated with human disease.
- The most famous of these is ***Corynebacterium diphtheriae***, the etiologic agent of diphtheria.

Corynebacterium diphtheriae

- *Corynebacterium diphtheriae* is an **irregularly staining, pleomorphic rod**.
- **Metachromatic granules** have been observed in rods stained with methylene blue.
- After overnight incubation, colonies can be seen on blood agar.
- More selective, differential media can be used to recover this pathogen from specimens in which other organisms are present.

Corynebacterium diphtheriae

- *C. diphtheriae* is the leading causing agent of **diphtheria**.
- It is a **non-motile, non-encapsulated, non-sporulating gram-positive rod-shaped bacterium**.
- Four subspecies are recognized:
 - *C. diphtheriae mitis*
 - *C. diphtheriae intermedius*
 - *C. diphtheriae gravis*
 - *C. diphtheriae belfanti*
- The four subspecies differ slightly in their colonial morphology and biochemical properties.

Corynebacterium diphtheriae

- *C. diphtheriae* produces **diphtheria toxin** which alters protein function in the host.
- The **diphtheria toxin gene** is encoded by a **bacteriophage** found in toxigenic strains, integrated into the bacterial chromosome.
- In addition to *C. diphtheriae*, two other corynebacteria species can produce diphtheria toxin and thus also cause diphtheria: ***C. ulcerans*** and ***C. pseudotuberculosis***. Both are zoonotic pathogens that can have the ability to produce diphtheria toxin.

Corynebacterium diphtheriae

- To accurately identify *C. diphtheriae*, a Gram stain is performed to show Gram-positive, highly pleomorphic organisms with no particular arrangement.
- Special stains like **Albert's stain** is used to demonstrate the **metachromatic granules** formed in the polar regions.
- An enrichment medium, such as **Löffler's medium**, is used to preferentially grow *C. diphtheriae*.

Corynebacterium diphtheriae

- A differential plate known as **tellurite agar**, allows all *Corynebacteria* (including *C. diphtheriae*) to **reduce tellurite to metallic tellurium**.
- The **tellurite reduction is colorimetrically indicated** by brown colonies for most *Corynebacteria* species or by a **black halo around the *C. diphtheriae* colonies**.

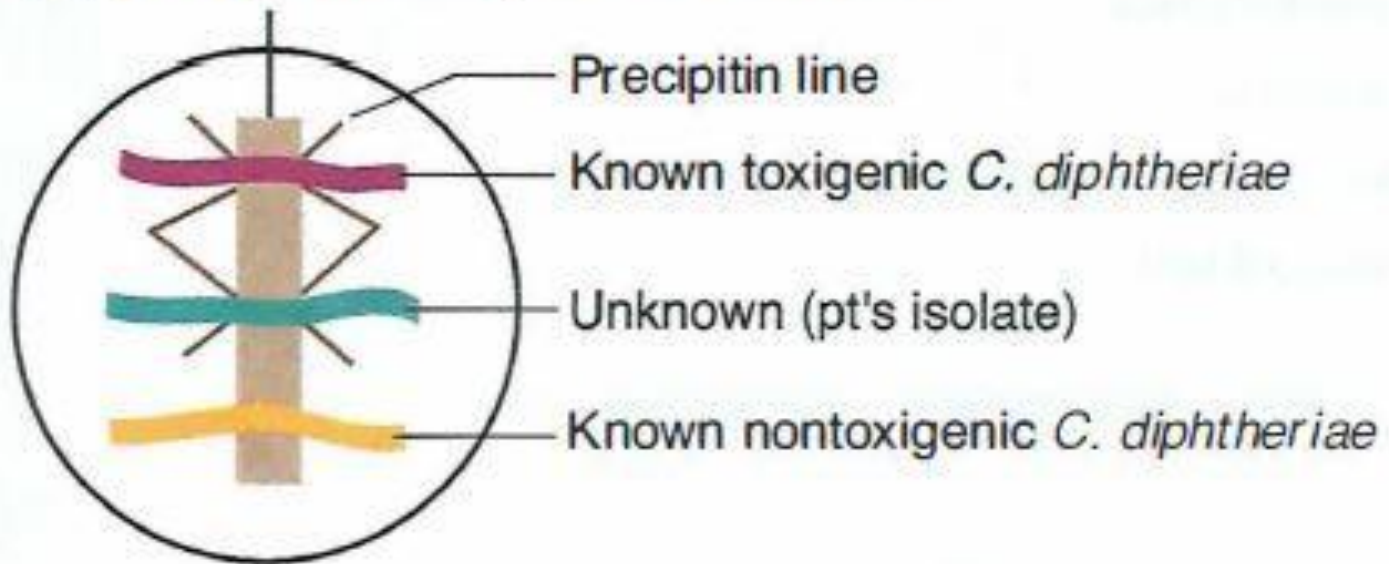
Corynebacterium diphtheriae

- Other diphtheroids do not have this ability with the exception of *C. ulcerans* and *C. pseudotuberculosis*.
- Organisms other than corynebacteria, such as staphylococci and streptococci, can reduce tellurite and produce black colonies.
- A low concentration of iron is required in the medium for toxin production. At high concentrations, iron molecules bind to an aporepressor on the beta bacteriophage, which carries the **Tox gene**. When bound to iron, the aporepressor shuts down toxin production.

Corynebacterium diphtheriae

- **Elek's test** for toxigenicity is used to determine whether the organism is able to produce the **diphtheria toxin**.

Filter paper strip with *C. diphtheriae* antitoxin



<https://microbeonline.com/elek-test-principle-procedure-results/>

Corynebacterium diphtheriae

- A strip of filter **paper impregnated with diphtheria antitoxin** is buried just beneath the surface of a special agar plate before the agar hardens.
- Strains to be tested are streaked with known **positive** and known **negative toxigenic strains** on the agar's surface in a line across the plate, and at a right angle to the antitoxin paper strip.
- After incubation for **24 hours at 37 °C** , plates are examined with transmitted light for the presence of fine precipitin lines at a 45-degree angle to the streaks. **The presence of precipitin lines indicates that the strain produced toxin that reacted with the antitoxin.**

Corynebacterium diphtheriae

- The portals of **entry** for *C. diphtheriae* are the **nose, tonsils, and throat**. The incubation period of diphtheria is 2–5 days (range: 1- 10 days).
- Once individual contracts the disease, they are **contagious for two weeks to a month**.
- Diseased individuals may experience sore throat, overall weakness, fever, and swollen glands.
- Diphtheria is **transmitted from human contact through respiratory droplets, such as coughing or sneezing**.

Corynebacterium diphtheriae

- Although the geographic occurrence for the disease is worldwide, **it is mainly to be found in tropical regions and underdeveloped countries.**
- Those facing the greatest risk of contracting the disease are immunocompromised individuals, poorly immunized adults, and unvaccinated children.
- When an **individual contracts diphtheria**, the only affected region of the body is the **upper respiratory system**. Diphtheria can cause a thick gray coating to build up in the throat or nose making it difficult to breathe and swallow.

Corynebacterium diphtheriae

For clinical purposes, it is convenient to classify diphtheria into a number of manifestations, depending on the site of disease:

- **Respiratory diphtheria**
 - Nasal diphtheria
 - Pharyngeal and tonsillar diphtheria
 - Laryngeal diphtheria
- **Cutaneous diphtheria**

Corynebacterium diphtheriae

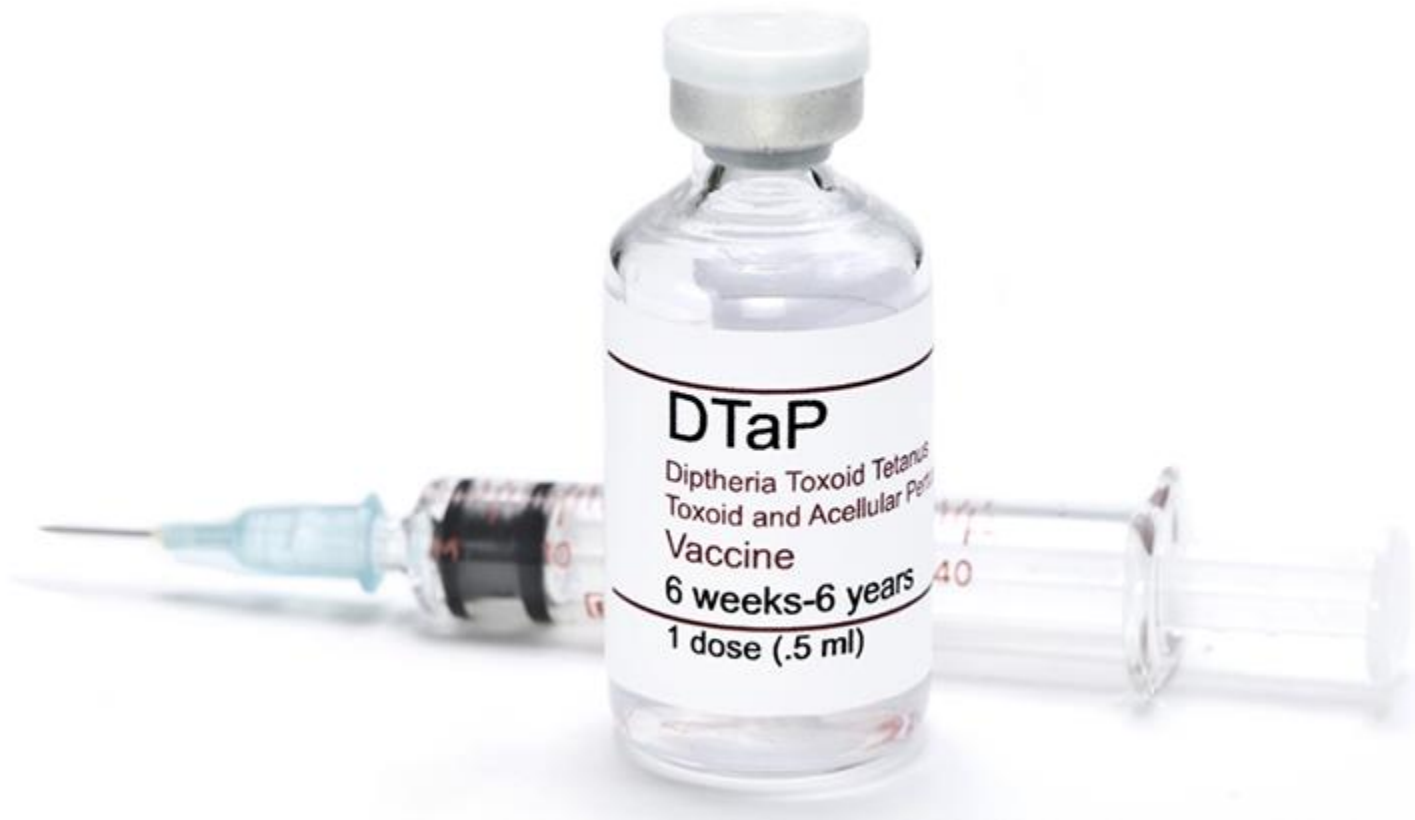
- Although unlikely, individuals can get sick from touching open sores or clothes that touched open sores of someone sick with the disease.
- **If untreated, the diphtheria toxin can get into the bloodstream causing damage to the kidneys, nerves, and heart.**
- Extremely rare but serious complications include suffocation and partial paralysis.

Corynebacterium diphtheriae

- After the provisional clinical diagnosis is made and appropriate cultures are obtained, persons with suspected diphtheria **should be given antitoxin and antibiotics in adequate dosage** and placed in isolation. **Respiratory support** and airway maintenance should also be administered as needed.
- The recommended antibiotic treatment for diphtheria is **erythromycin** orally or by injection for 14 days, or procaine **penicillin G** daily, intramuscularly for 14 days.

Corynebacterium diphtheriae

- One preventive measure against diphtheria would be to get the DTaP vaccine if available.



<https://childrenshealthdefense.org/news/one-in-nine-adverse-events-reported-after-dtap-vaccination-is-serious-but-cdc-says-dont-worry-be-happy/>