## Gram positive Rods

### CORYNEBACTERIA

- small, slender, pleomorphic, gram-positive rods. They are nonmotile and unencapsulated, and they do not form spores.
- Corynebacterium is a large genus of diverse habitat. Most species are facultative anaerobes, and those associated with humans, including the pathogen *C. diphtheriae*, grow aerobically on standard laboratory media such as blood agar.
- *C. diphtheriae* is found in the throat and nasopharynx of carriers and in patients with diphtheria.
- Clinical significance: Infection may result in one of two forms of clinical disease, respiratory or cutaneous, or in an asymptomatic carrier state, (a. Upper respiratory tract infection, b. Cutaneous diphtheria)
- Diphtheria is a local infection, of the throat, and the organism is primarily spread by respiratory droplets, usually by convalescent or asymptomatic carriers.
- Diphtheria is caused by the local and systemic effects of a single exotoxin that inhibits eukaryotic protein synthesis. The toxin molecule is a heat-labile polypeptide that is composed of two fragments, A and B. Fragment B binds to susceptible cell membranes and mediates the delivery of fragment A to its target.

The infection produces a distinctive thick, grayish, adherent exudate (pseudomembrane) that is composed of cell debris from the mucosa and inflammatory products. It coats the throat and may extend into the nasal passages or downward in the respiratory tract, where the exudate sometimes obstructs the airways, even leading to suffocation. As the disease progresses, generalized symptoms occur caused by production and absorption of toxin. Although all human cells are sensitive to diphtheria toxin, the major clinical effects involve the heart and peripheral nerves.

Cutaneous (skin) diphtheria which usually develops when *C. diphtheriae* infects open wounds. Infection of the skin rarely leads to the serious complications associated with diphtheria of the throat.

### LABORATORY FEATURES

Specimens: Include throat, and, or nasopharyngeal swabs to confirm a diagnosis of throat diphtheria, and a skin swab if cutaneous diphtheria is suspected

Morphology

C. diphtheriae is Gram positive but usually stains unevenly and weakly. It is markedly pleomorphic. Long, thin, and curved forms can be seen and

also short rods and rods enlarged at one end (club shaped). They often appear in clusters, joined at angles like Chinese letters Commensal diphtheroids: These are strongly Gram positive and stain uniformly. They are usually short and show little variation in size and form.

***Volutin granules***

In Albert stained smears, particularly from Loeffler serum or Dorset egg cultures, *C. diphtheriae* often appears beaded due to the presence of dark staining granules in the rods.

These granules, known as volutin or metachromatic granules, are energy-storing inorganic polyphosphate units. In some strains the granules form at the ends of the rods. In toluidine blue stained smears, the organisms stain pale blue and the granules dark red-purple.

**Culture**

***Loeffler serum medium and Dorset egg medium*:** *C. diphtheriae* grows rapidly on these media, producing significant growth in 4–6 hours. The characteristic morphological features of *C. diphtheriae*, especially granule formation, are well developed.

***Note:*** It is not advisable to use either Dorset egg or Loeffler serum medium as a primary medium for isolating *C. diphtheriae* because commensal diphtheroids may overgrow the diphtheria bacteria.

***Tellurite blood agar*:** This medium is widely used as a primary medium for isolating *C. diphtheria* from throat and nasopharyngeal swabs. *C. diphtheriae* reduces tellurite and produces grey or grey-black colonies measuring 0.5–2 mm in diameter after 24–48 h incubation

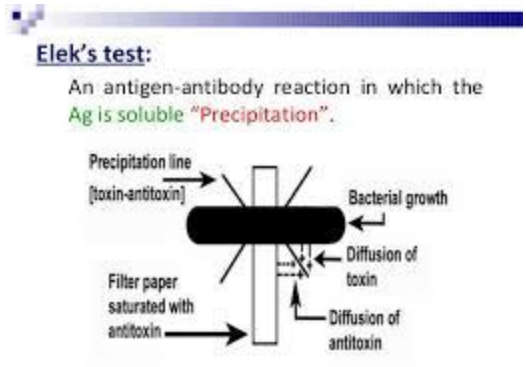
***Tinsdale medium:*** After 24–28 h incubation, *C. diphtheriae* colonies are grey-black, raised, and surrounded by a dark brown area

**Biochemical tests**

- Catalase and nitrate positive.
- Oxidase negative.
- Urease negative

**Toxigenicity (virulence) testing of *C. diphtheriae***

Diphtheria is caused by toxin-producing strains of *C. diphtheriae*. Toxigenicity of *C. diphtheriae* can be tested by the Elek gel precipitation test.




***Listeria***

Listeria species are slender, short, gram-positive rods *with some bacteriologic features that resemble* those of both corynebacteria and streptococci. They do not form spores. Sometimes they occur as diplobacilli or in short chains. Listeria species are **catalase-positive**, and display a distinctive **tumbling motility** by light microscopy in liquid medium, which is most active after growth at 25°C. These characteristics distinguish it from Streptococcus (catalase negative) or Corynebacterium (nonmotile) species, both of which may be confused morphologically with Listeria. Listeria species grow on a variety of enriched media.

Listeria monocytogenes is the only species that infects humans

Listeria infections, which may occur as sporadic cases or in small epidemics, are usually foodborne

Most cases occur at the extremes of life (eg, in infants less than 1 month of age or adults over 60 years of age).

Dairy product outbreaks have been traced to post-pasteurization contamination or deviation from recommended time and temperature guidelines. An important feature of some epidemics has been the ability of *L. monocytogenesto grow at refrigerator temperatures,* allowing scant numbers to reach an infectious dose during storage.

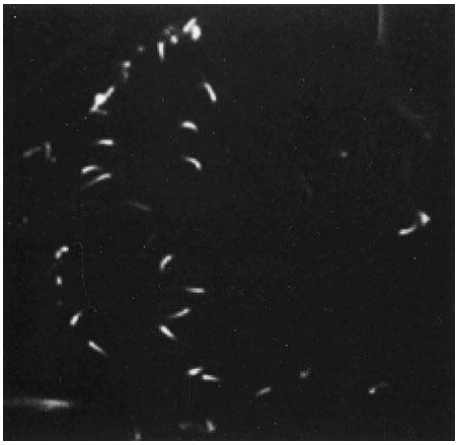
*L. monocytogenesmay also be transmitted transplacentally to the fetus, presumably* following hematogenous dissemination in the mother. It may also be transmitted to newborns in the birth canal in a manner similar to group B streptococci.

Pathogenesis

L. monocytogenes is an intracellular parasite.

1. Listeria is phagocytosed by Macrophage and incorporated into a phagolysosome.

2. The bacterial product listeriolysin O lyses the phagolysosome, allowing the escape of listeria.
3. Listeria multiplies and assembles an actin filament tail that pushes the bacterium to the surface of the macrophage.
4. Apseudopod extension froms facilitating transfer of the listeria into another phagocyte.



Intracellular movement of *Listeria monocytogenes. L. monocytogenes* cells are shown within infected cells in culture. The immunofluorescent stain used an antibody that binds to actin, demonstrating the **comet-like actin"tails,"** which trail the bacteria as they move through the cell.

**Listeriosis(peaks in summer)**
Healthy adults and children: generally asymptomatic or diarrhea with low % carriage.
**Pregnant women**: symptomatic carriage , septicemia characterized by fever and chills. Can cross the placenta in septicemia.
**Neonatal disease:**

- Early onset (granulomatosis infantiseptica) in Uterotransmission, sepsis with high mortality, disseminated granulomas with central necrosis.
- Late onset: 2-3 weeks after birth from fecal exposure, meningitis with septicemia.

**Immunocompromised patients**: septicemia and meningitis.

**Laboratory identification**
The organism can be isolated from blood, cerebrospinal fluid, and other clinical specimens by standard bacteriologic procedures. On blood agar, *L. monocytogenes* produces a small colony surrounded by a narrow zone of β hemolysis
*Listeria* species can be distinguished from various streptococci by morphology, motility, and the production of catalase.

**BACILLUS SPECIES**
gram-positive, form endospores, and are either strict aerobes or aerotolerant anaerobes (that is, they can grow in the presence of oxygen, but do not require it).

Most of the species of Bacillus are found in soil and water and are usually encountered in the medical laboratory as airborne contaminants.
B. anthracis, the cause of the disease anthrax, is clinically the most important member of this genus.

**A. Bacillus anthracis**

- Form very long chains of rods and in culture is nonmotile and nonhemolytic colonies are characterized by a rough, uneven surface with multiple curled extensions at the edge resembling a "Medusa head."
- *B. anthracis* has a D-glutamic acid polypeptide capsule of a single antigenic type that has antiphagocytic properties.
- The organism is also is a potent producer of one or more exotoxins, which although they have been given multiple names (lethal factor, edema factor, protective antigen), represent separate activities of a protein complex. In various combinations and configurations these proteins may exhibit binding, cytolytic, or enzymatic activity.

**Clinical significance**

**a. Cutaneous anthrax:**
Upon introduction of organisms or spores that germinate, a papule develops. It rapidly evolves into a painless, black, severely swollen "malignant pustule,"

**b. Pulmonary anthrax** (**Woolsorter's disease**)**:** Caused by inhalation of spores

**c. Enteric anthrax**: A severe form of gastroenteritis with fever, abdominal pain and bloody diarrhoea, due to ingesting infected meat. Septicaemia often develops.

**d. Meningoencephalitis**: Usually as a complication of septicaemia and occasionally as primary anthrax meningoencephalitis.

## DIAGNOSIS

***Caution***: *B. anthracis* is a high risk infectious pathogen, therefore handle specimens and infected material with care, wearing protective gloves and face mask, and following recommended safety procedures. **Use 4% v/v formalin** solution to decontaminate infected material and laboratory ware.

**Sample**: skin lesions, sputum, blood, and CSF are the primary means of anthrax diagnosis.

**Morphology**

Gram positive (or Gram variable) non-motile bacillus, often appearing joined end to end in chains.

*In smears from specimens*: Bacilli are capsulated. The capsular material often appears irregular and fragmented. When stained using Loeffler's polychrome (McFadyean) methylene blue, the bacilli stain blue and the

capsular material stains purple-pink. Alternatively, **Giesma stain** can also be used when MacFadyean methylene blue is not available.
*In smears from aerobic cultures*: Bacilli are non-capsulated but contain oval spores (same diameter as the bacilli), giving the organisms a beaded appearance.

**Fixation of smears**

*B. anthracis* is not killed by heat-fixation. Smears should be chemically fixed by immersing the dry smears in a container of **potassium permanganate** 40 g/l solution for 10–15 minutes.
***Important:*** When organisms resembling *B. anthracis* are seen in smears, specimens should be sent for further testing to the nearest Public Health Laboratory and public health officials notified as soon as possible

**Culture**

*B. anthracis* grows aerobically and anaerobically (facultative anaerobe). The temperature range for growth is 12–45 ºC with an optimum of 35–37 ºC. Spore formation is best between the range 25–30 ºC.

***Blood agar:*** *B. anthracis* produces large 2–5 mm in diameter, grey-white, irregular colonies with wavy edges. The colonies are nonhaemolytic or only slightly haemolytic. The saprophytic species are usually β-hemolytic and motile; these features can be used to exclude *B. anthracis.*

***Gelatin stab culture***

The organism slowly liquefies the gelatin.

**Prevention**

Pasteur’ s vaccine used a live strain attenuated by repeated subculture that resulted in the loss of a plasmid encoding toxin production.

**B. Other bacillus species**

A commonly identified species is Bacillus cereus. Strains of this species produce a tissue-destructive exotoxin. B. cereus also causes food poisoning by means of enterotoxins with either emetic or diarrheal effects.
The toxin is produced when the bacilli sporulate, usually in rice or other cooked food and then stored in warm temperatures.
*B. cereus* unlike *B. anthracis* is motile, non-capsulate, and produces haemolytic colonies on blood agar. On egg-yolk agar, *B. cereus* gives a strong lecithinase reaction. It rapidly liquefies gelatin stabs.
Mannitol egg-yolk phenol-red polymyxin agar (MYPA) is recommended as a selective medium for the isolation of *B. cereus* from faeces, vomit, or food. After overnight incubation at 35–37 ºC, large 3–7 mm flat, dry grey-white colonies surrounded by an area of white precipitate are produced.

*B. cereus* produces *beta*-lactamase and is resistant to penicillin (B. Anthracis is sensitive) and cephalosporins. Antimicrobials with activity against *B. cereus* include gentamicin, erythromycin, vancomycin and clindamycin.

**Further Readings:**

***SHERRIS MEDICAL MICROBIOLOGY:* AN INTRODUCTION TO INFECTIOUS DISEASES**, 4TH EDITION *by KENNETH J. RYAN, MD C. GEORGE RAY, MD*

**Lippincott's Illustrated Reviews: Microbiology**, Third Edition, Copyright © 2013 Lippincott Williams & Wilkins, a Wolters Kluwer business.

**District Laboratory Practice in Tropical Countries**, Part 2, Second Edition by Monica Cheesbrough, Cambridge University Press

**CASES IN MEDICAL MICROBIOLOGY AND INFECTIOUS DISEASES**, FOURTH EDITION, ASM Press Washington, DC, by Peter H. Gilligan, Ph.D.
Daniel S. Shapiro, M.D. and Melissa B. Miller, Ph.D.