Spore-Forming Gram-Positive Bacilli

Bacillus and *Clostridium* Species

Bacillus

The genus *Bacillus* includes large aerobic, gram-positive rods occurring in chains. Most members of this genus are saprophytic organisms prevalent in soil, water, and air and on vegetation, such as *Bacillus cereus* and *Bacillus subtilis* . Some are insect pathogens, such as *B thuringiensis* *B cereus* can grow in foods and cause food poisoning by producing either an enterotoxin (diarrhea) or an emetic toxin (vomiting). Both *B cereus* and *B thuringiensis* may occasionally produce disease (eg, meningitis, endocarditis, endophthalmitis, conjunctivitis, or acute gastroenteritis). *B anthracis*, which causes **anthrax,** is the principal pathogen of the genus.

Morphology and identification

Typical cells, measuring 1 × 3–4 μm, have square ends and are arranged in long chains; spores are located in the center of the nonmotile bacilli. Colonies of *B anthracis* are round. Gelatin is liquefied, and growth in gelatin stabs resembles an inverted fir tree.

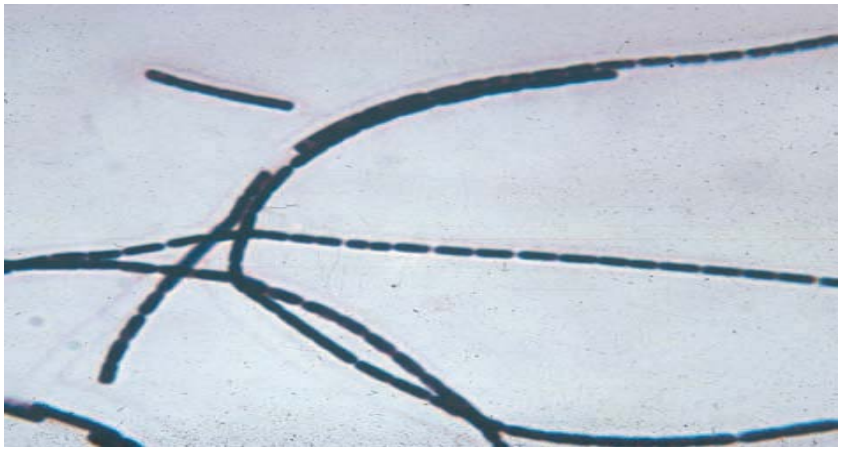
Bacillus anthracis

Pathogenesis

Anthrax is primarily a disease of goats, sheep, cattle, horses(specially herbivores) .Human become infected incidentally by contact with infected animals or their products.Infection is usually acquired by the entry of spores through injured skin (cutaneous anthrax) or rarely the mucous membranes (gastrointestinal anthrax) or by inhalation of spores into the lung (inhalation anthrax). Bacilli spread via lymphatics to the bloodstream, and they multiply freely in the blood and tissues shortly before and after the animal's death. Antiphagocytic capsule and Anthrax toxins are a major virulence factors and cause of death.

In Cutaneous anthrax a pruritic papule develops 1–7 days after entry it resembles an insect bite, and a necrotic ulcer develops with central black eschar. Lymphadenopathy and systemic signs and symptoms of fever, malaise, and headache may occur.

, cutaneous anthrax can lead to sepsis, the consequences of systemic infection—including meningitis—and death.
Inhalation anthrax may be as are associated with marked hemorrhagic necrosis .



Diagnostic Laboratory Tests

-Specimens are fluid or pus from a local lesion, blood, pleural fluid, CSF, stool . Stained smears show chains of large gram-positive rods.
-Culture on blood agar, nonhemolytic gray to white, a rough texture and a ground-glass appearance. Medusa head, “curled hair” may project from the colony.
-Demonstration of capsule on bicarbonate-containing medium in 5–7% carbon dioxide.
- ELISA, PCR, Phage and other tests

Treatment

Ciprofloxacin
penicillin G, along with gentamicin or streptomycin

Bacillus cereus

Present in soil, vegetables , food and poultry products. It has been considered as causative agent of food poisoning in humans.

Morphology

Gram-positive bacilli which have straight or rounded ends, spores forming . Motile by means of peritrichous flagella.

Culture Characters

Aerobic , large, irregular pale colonies

Antigenic factors and Pathogenicity

-Somatic Ag, Flagella
-Virulence factors : Phospholipase *C* , Haemolysins, Diarrhoeal enterotoxin.

Clinical Features

Food poisoning

Laboratory Diagnosis

faeces and vomitus are inoculated on selective mannitol-phenol red-egg yolk-polymyxin medium and typical colonies studied and further examined. β-hemolysis on blood agar, motile form(swarming) in semisolid media

Clostridium

Thick, Gram-positive, sporing rod bacteria, strictly anaerobic. Their natural habitat is the soil and the intestinal tracts of humans and animals. With the exception of C. perfringens, clostridia are flagellated.
C. perfringens colonies are convex, smooth, and surrounded by a hemolytic zone. Colonies of motile clostridia have an irregular, ragged edge.

Clostridium perfringens

Morphology and culture

Nonmotile bacilli with blunt or square ends. The spores are large, oval and subterminal. Beta haemolysis on blood agar.These organisms grow rapidly in cooked meat medium and produce lot of gas.

Pathogenecity

One of the pathogens that cause Gas Gangrene (Clostridial Myonecrosis) The toxins produced show necrotizing, hemolytic, and/or lethal activity. They also produce collagenases, proteinases, DNases, lecithinases, and hyaluronidase, all of which destroy tissue. they frequently contaminate openwounds, often t with other microorganisms.

Gas gangrene an aggressive infection of the musculature with myonecrosis and toxemia.

Diagnosis

-Clinical samples. Collect sample from the deeper recesses of the wound Pus, excised tissue or necrosed tissue should be preferred over swab. Cooked meat medium preferred to avoid killing during transmit.
-Stain smear ,gram-positive bacilli
-Culture. Cooked meat broth, blood agar with neomycin should be Incubated with 10% carbon dioxide.

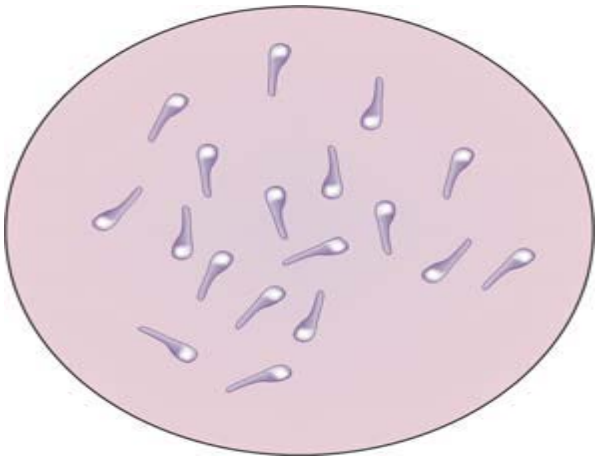
Treatment

Primary treatment is surgical, removal of dead tissue from the wound with antitoxin accompanied by antibiotics (penicillins, cephalosporins). Treatment with hyperbaric O2 in special centers has proved effective: patients breathe pure O2 through a tube or mask several times during two-hour periods.

Clostridium tetani

Morphology

Long rod,thin and straight with rounded ends. Gram positive. In older cultures and smears from wounds, the bacilli are usually Gram negative. A spore terminally located and greater diameter than the vegetative cell, giving the characteristic drum stick appearance. No capsule. Motile by peritrichate flagella



Pathogenicity

Tetanus (lockjaw) is an acute disease, caused by a strong neurotoxin. Tetanospasmin (an AB toxin) , binds specifically to neuron receptors. and is responsible for proteolysis in the anterior horns of the spinal cord.

Diagnosis

-Clinical specimen. Wound exudate or tissue removed from the wound.
-Direct smear shows few drum stick appearance bacilli.
-Direct immunofluorescence test
-Culture examination. Cooked meat medium as well as blood agar. Plates are incubated anaerobically .At 80°C spores of tetanus bacilli get killed.
-Toxin detection by pathogenicity in an animal test (mouse) or detection of the toxin gene with PCR.

Treatment

Antitoxic therapy with immune sera is applied .

Clostridium botulinum

Rods straight with rounded ends. Spores are near the ends and oval in shape . Motile peritrichous flagella. These organisms do not have capsule.

Pathogenicity

Foodborne **botulism** is not an infection, but , the toxin is ingested with food. Infant botulism involves ingestion of spores and wound botulism results from infection of a wound. Their neurotoxin is a heat-labile protein.
The toxin is absorbed in the gastrointestinal tract, and
then transported to the peripheral nervous system in the bloodstream. Within a matter of hours or days paralysis symptoms occur, especially in the nerves of the head.

Diagnosis

-Clinical samples**.** With extreme precautions specimens that can be collected shall include feces, food, vomitus, gastric fluid, serum, and occasionally wound exudates.
-Fluorescent antibody procedure.
-Culture**.** On egg-yolk agar, blood agar and two bottles of cooked meat broth, incubated anaerobically at 30°C and incubated for 3-5 days. In
-Neutralisation test in mice
.

Treatment

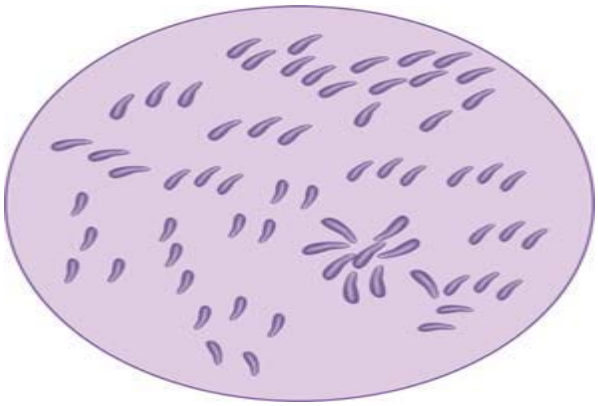
Urgent administration of antitoxin.

Non Spore-Forming Gram-Positive Bacilli

Corynebacterium diphtheriae

Morphology

Gram-positive, non-motile and non-sporing bacilli. It is the causative agent of **diphtheria**. The organism a pleomorphic. “club-shaped” appearance, with **metachromatic granules**



Culture

It requires serum for their growth. In liquid medium a pellicle forms on the surface .The colonies are small, circular, creamy and glistening.
It can grow on Blood Tellurite Agar Medium as(selective medium) .

The pathogenicity

Classically the site of infection is nasopharynx. Type AB exotoxin causes local necrosis, with inflammatory response leads to form pseudo-membrane which is white to grey on tonsils, larynx and trachea , then absorption of toxin can result in striking cervical adenopathy (bull neck), damage to the protein synthesis, neural and cardiac involvement , toxaemia , which may lead to death.

Laboratory Diagnosis

-Sample Collection and identification
Two smears are prepared using one of the throat swabs on two slides. One smear is stained with Gram's stain and the other with Albert's Stain, they shows; Thin, slender, gram-positive bacilli with clubbing at ends, Metachromatic granules , Bacilli arranged at acute angles giving *Chinese letters*
-Isolation of organism on different culture media
-Confirmation of toxigenicity in vitro and in vivo

Treatment

-Using diphtheria anti-toxin
-Penicillin and erythromycin

Mycobacterium

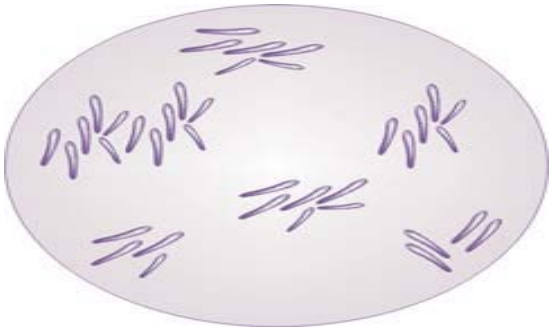
They are named so because of the mold like pellicular growth in liquid medium (*myco:* fungus). They have the ability to resist decolourisation by a weak mineral acid after staining with one of the aryl-methane dyes. This gives the name *acid fast bacteria* to these organisms.
They can be classified in to 5 groups, some of them are:

Group 1. Obligate pathogens
— *M. tuberculosis*
— *M.leprae*
— *M. bovis*
Group 2. Skin pathogens
— *M. marinum*
— *M. ulcerans*

Mycobacterium tuberculosis

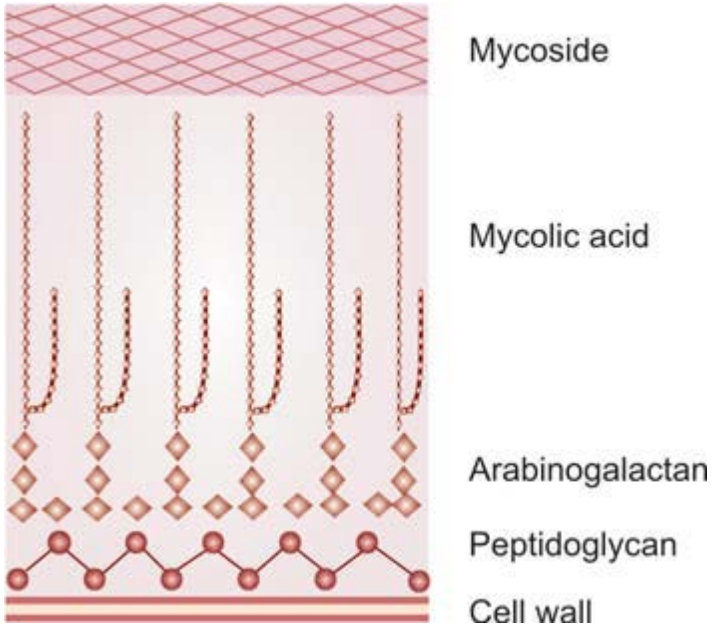
Morphology

are slender, straight or slightly curved rod shaped organisms occurring singly, in pairs or in small groups, non-sporing, non-motile and non-capsulated. Gram positive though they do not take the stain readily. These organisms resist decolourisation by 25% sulphuric acid and absolute alcohol for ten minutes and hence these are called acid and alcohol fast.



Mycobaterial Cell Wall

It has high lipid content which accounts for about 60% of the dry weight of the cell.



Pathogenesis

Tuberculosis: The most frequent portal of entry is lungs, resulting from the inhalation of airborne droplets containing a few bacilli
Or less, through drinking contaminated milk to the intestine, or skin.
Pathology of tuberculosis consists of lesion, called the tubercle.
Dissemination of bacilli from the site of implantation occurs via the lymphatics to the regional lymph nodes .
Infection with *M. tuberculosis* induces delayed hypersensitivity (allergy) and resistance to infection (immunity).

Laboratory Diagnosis

-Clinical Sample: In pulmonary tuberculosis, sputum is the sample of choice. If any other organ is suspected, sample has to pertain specific organ or system such as urine for renal tuberculosis and cerebrospinal fluid for tubercular meningitis. CSF and pus are placed directly in sterile containers and sent to the laboratory without any delay.

-Direct demonstration by microscopy: selecting a purulent portion of sputum , smears are air dried, fixed and stained with Ziehl-Neelsen
Acid fast bacilli are seen as bright-red rods.
(At least some 100,000 tubercle bacilli must be present in per ml of sputum , it is essential that at least 100 fields are examined)
-Fluorescent Staining
-Petroff's Method

-Isolation by culture: Most commonly used medium is Lowenstein Jensen (LJ) medium.

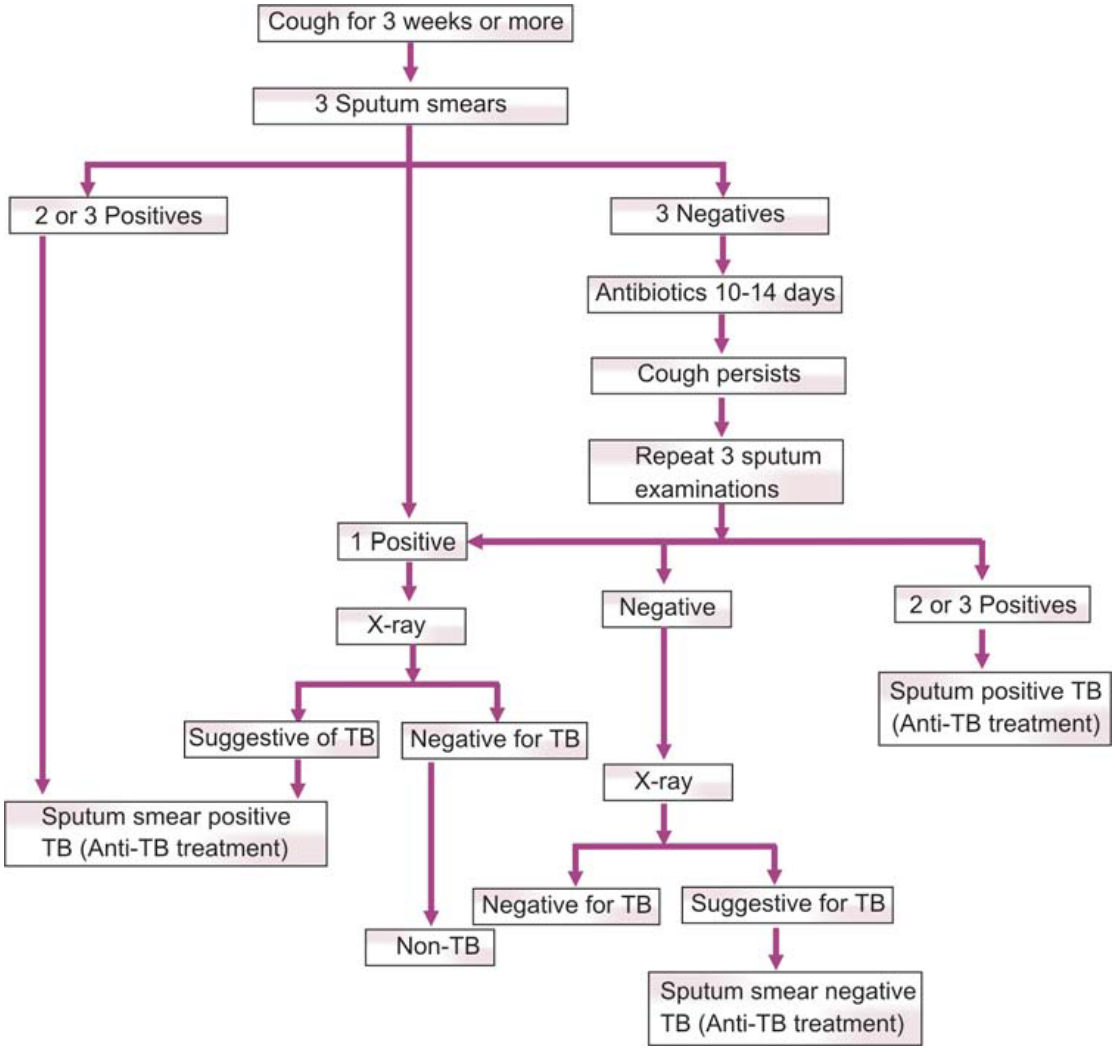
- Biochemical tests: eg; it is positive for niacin and urease

-Animal pathogenicity

- Immunodiagnosis and Molecular techniques: EISA, PCR

-Tuberculin test

- Histopathological examination: by taking biopsy



Treatment

Table 4.4 Scheme for Chemotherapy of Tuberculosis

	Standard scheme	Months	Short scheme *	Months
Initial phase	isoniazid (INH) rifampicin (RMP) ethambutol (EMB)	2	isoniazid rifampicin ethambutol pyrazinamide (PZA)	2
Continuation phase	isoniazid rifampicin	7	isoniazid rifampicin	4

Mycobacterium lebrae

Morphology

Are straight or slightly curved rodlike bacteria pointed, rounded or club-shaped ends, non sporing.Gram-positive, it is acid fast but less strongly acid fast than *M. tuberculosis*. 5% sulphuric acid is employed for decolourisation after staining with carbol fuchsin. In stained skin smears , they are seen as pink lines, in bright pink compact masses known as *globi*.

Cultivation

It has not been possible to culture *M. leprae* in bacteriological media or tissue cultures. It can be grown in the footpad of a mouse to some extent, for determining viability in tissue biopsies and for performing drug sensitivity tests.

Clinical Features

The bacterium causes **leprosy** (Hansen's disease).

Transmission:

• *M. leprae* is not highly infectious, it may be occurred through inhalation of infectious organisms
• Infectious people are thought to shed the organism from the nasal mucous membranes, especially if there is ulceration, and can survive in for more than 36 hours in nasal scretions

Diagnostic signs of leprosy are:
a. Inability to feel touch, heat and/or pain in the affected area.
b. Enlargement and/or tenderness of peripheral nerves associated with sensory loss and/or paralysis.
c. Finding non-cultivable bacilli in skin smears taken from the affected areas.
Some types of leprosy patient presents with nodular lesions (lepromas) containing many acid fast *M. leprae* bacilli.

An obligate intracellular pathogen that requires the environment of the host macrophage for survival and propagation. Estimates of the replication rate *in vivo* are on the order of 10 to 12 days. Tuberculoid

leprosy is characterized by self-healing granulomas containing only a few, if any, acid-fast bacilli.

Laboratory Diagnosis

-Demonstration in smears*:* The diagnosis consists of demonstration of acid fast bacilli in the lesions. It is demonstrated
in “slit-skin smears” or in skin biopsies with Z-N staining,
-Skin testing (Lepromin test)
-PCR: Recently PCR for defection of M. leprae DNA

Treatment

Multi-drug therapy or MDT , : dapsone, rifampin, clofazimine,
The disease can be treated in 6 months, leprosy may require primary treatment for 3 years.