Chapter 17

Gram positive aerobic rods

There are **four** medically important genera of Gram positive rods:

Bacillus, Clostridium, Corynebacerium and ***Listeria***.

Bacillus, and ***Clostridium*** are **spore forming**.

Bacillus is **aerobic** and ***Clostridium*** is **anaerobic**.

AEROBIC NON SPORE FORMING GRAM POSITIVE RODS

There are **two** important pathogens:

1. ***Corynebacterium diphtheriae***

2. ***Listeria monocytogenes***

Corynebacterium diphtheriae

They cause **diphtheria**. Other species (diphtheroids) are opportunistic pathogens.

Important properties

-- *Corynebacteria* are **Gram-positive, aerobic, non motile, rod-shaped** bacteria.

-- They **do not form spores** or branch but they have characteristic irregular, club-shaped rods arranged in **V shapes**, **Chinese letters** or **palisades**.

-- These rods have **beads** which contain pyrophosphate and **stain metachromatically.**

Note the Chinese letter arrangement in the encircled areas

Transmission

-- Humans are the **only natural hosts**.

-- Transmission is by **airborne droplets**.

-- It may **infect a preformed skin lesion**.

Pathogenesis

-- **Invasiveness** is pre requirement for **exotoxin** production.

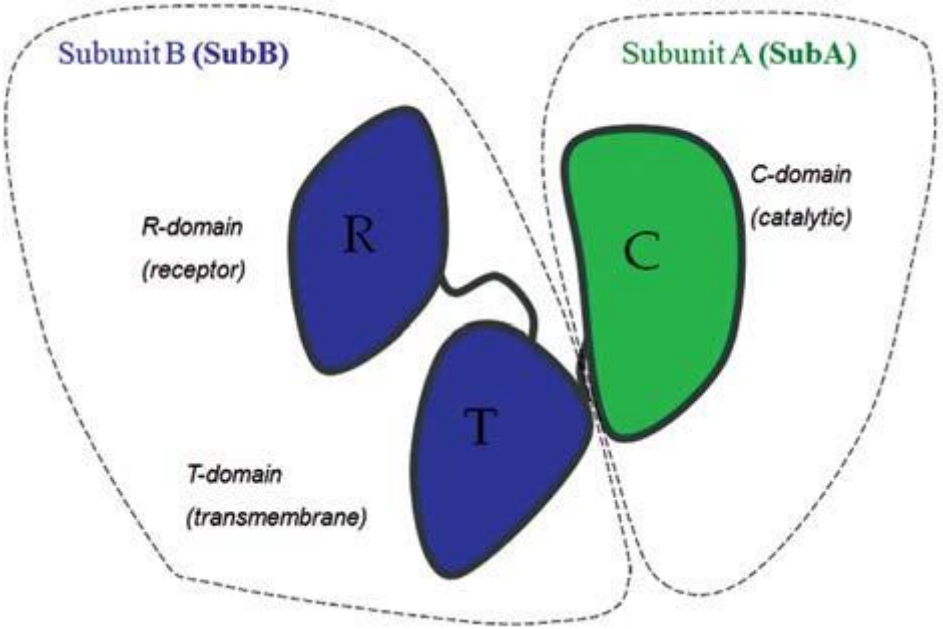
-- **Diphtheria toxin inhibits protein synthesis by ADP-ribosylation** of elongation factor 2 (**EF-2**) and all eukaryotic cells are affected due to interference in protein synthesis.

-- **It doesn't affect prokaryotes.**

Invasiveness

-- The diphtheria toxin (DTx) is a **two-component exotoxin** containing an A and B domains.

-- Fragment A catalyzes the transfer of ADP-ribose from NAD.



-- Attachment of the ADP ribosyl group occurs at an unusual derivative of histidine called **diphthamide**.

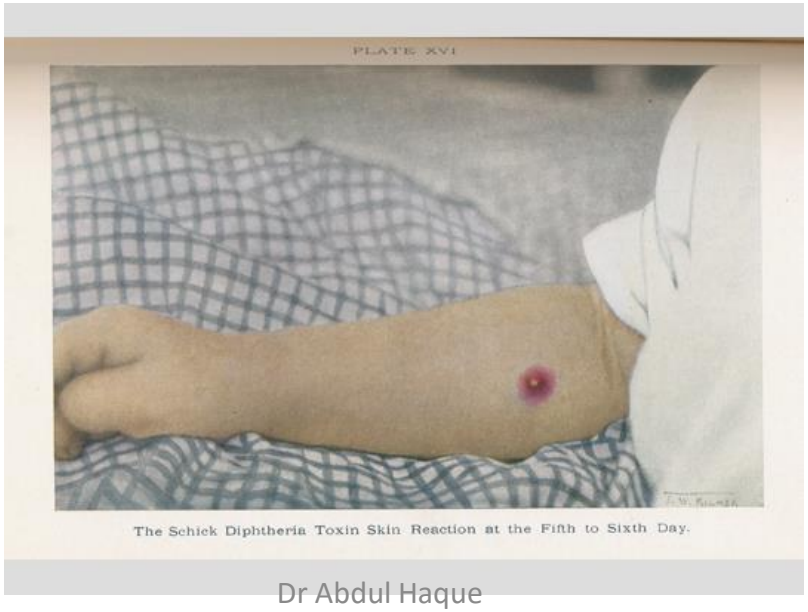
-- Relevant genes are located on a **temperate phage** called **β phage**.

-- During lysogenic phage, these genes integrate into bacterial chromosome and toxin is synthesized.

Host response consists of following:

1. Local inflammation in the throat with a **fibrinous exudate** which forms **pseudomembrane**.

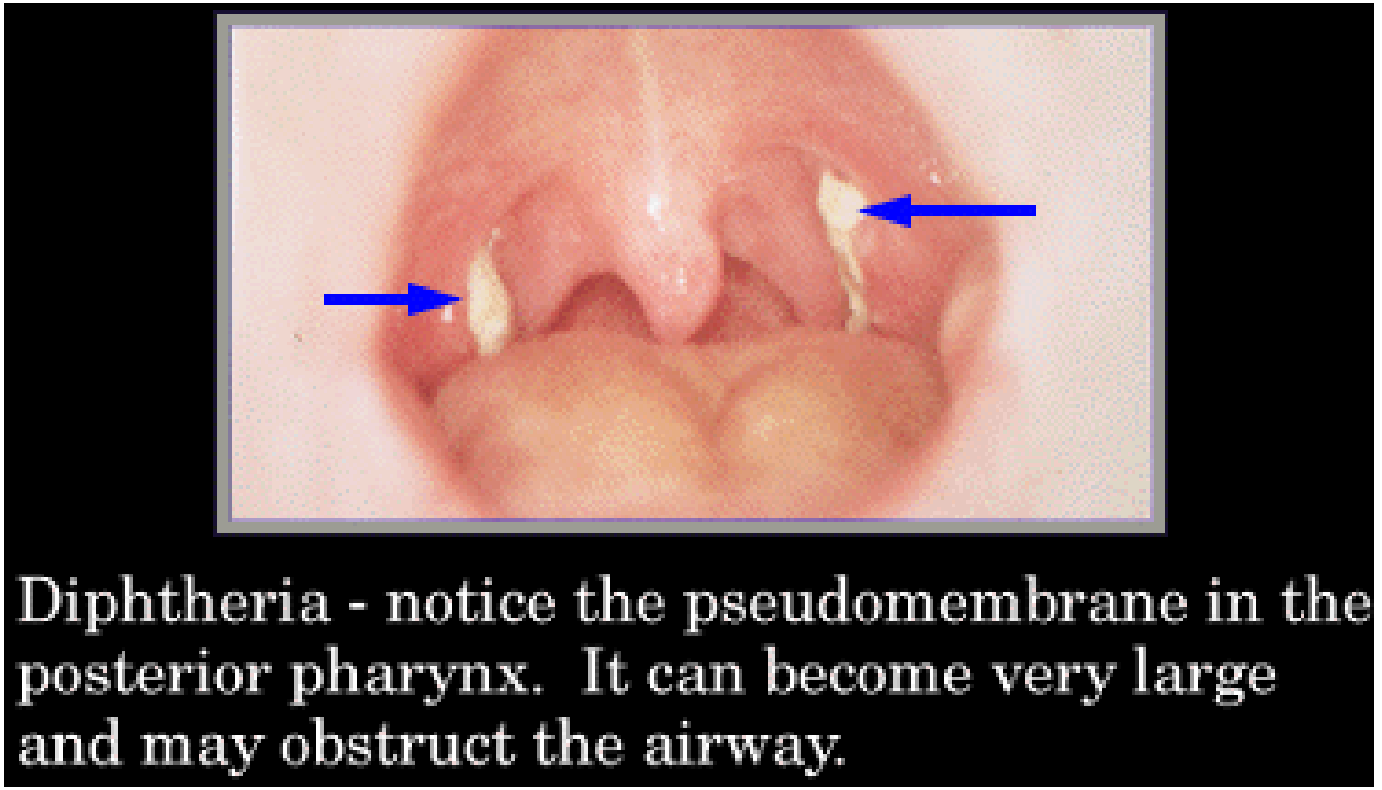
2. An antibody is produced that neutralizes exotoxin by blocking binding sites. Immune status is checked by **Schick's skin test** which is positive if no antibody is present.



Clinical findings

Thick, grey, adherent **pseudomembrane** over the **tonsils** and **throat** is the major finding.

Non specific symptoms include **fever, sore throat**, and **cervical adenopathy**.



Diphtheria - notice the pseudomembrane in the posterior pharynx. It can become very large and may obstruct the airway.

There can be **three** complications:

1. Extension of membrane into larynx and trachea causing **airway blockage**.

2. **Myocarditis** accompanied by arrhythmias and circulatory collapse.

3. Nerve weakness or **paralysis** esp. of cranial nerves. Paralysis of muscles of soft palate and pharynx may lead to regurgitations through nose.

-- **Cutaneous diphtheria** causes ulcerating skin lesions covered by a gray membrane.

Cutaneous type

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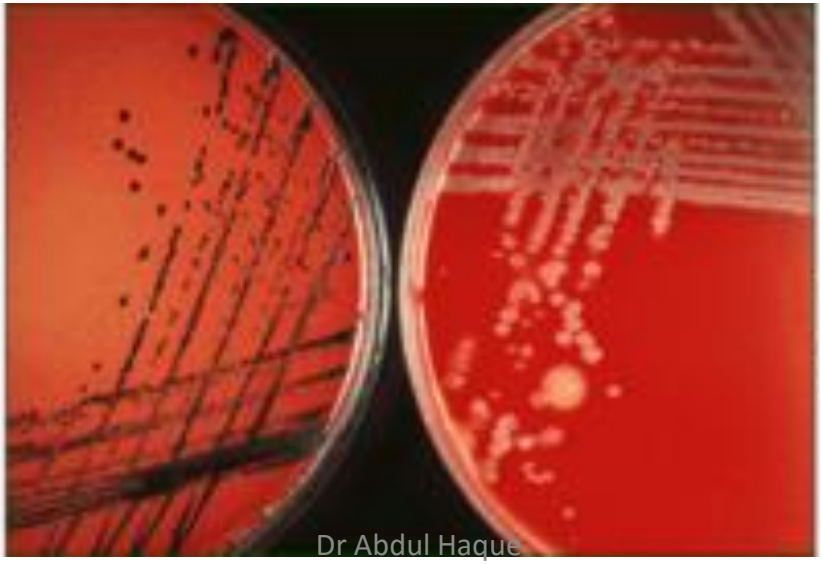
Laboratory diagnosis

-- It involves **isolation of organism** and demonstration of **toxin production.**

-- **Antitoxin treatment should not wait for laboratory results**.

-- A throat swab is cultured on **Loeffler's medium**, **tellurite plate** and **blood agar** plate.

-- **Typical gray black color** of tellurium in colonies is diagnostic.



-- If culture is positive, **animal inoculation**, **antibody based precipitin test** and **PCR** may be used for detection of toxin or its gene.

-- Although it cannot be diagnostic, smears stained with **Gram's method** and **methylene blue** (showing **metachromatic granules**) can be suggestive.

Treatment

-- **Antitoxin should be given immediately** on basis of clinical picture without waiting for laboratory reports.

-- The toxin binds rapidly and irreversibly to cells and, once bound, cannot be neutralized by antitoxin.

-- Because of **horse origin** of antitoxin, **hypersensitivity** should be checked and treatment for anaphylaxis must be available. Serum sickness may occur as well.

-- Antibiotics such as **Penicillin G** or **erythromycin** can play a secondary role by inhibiting bacterial growth and thus reducing toxin production and to decrease incidence of carriers.

Prevention

-- **Diphtheria toxoid**, prepared by treating exotoxin with formaldehyde, is used for immunization.

-- **Three doses** are given at 2, 4, and 6 months of age with boosters at 1 and 6 years age.

-- A **booster** every 10 years is recommended.

-- Immunization **does not** prevent nasopharyngeal carriage of the organism.

Listeria monocytogenes

-- ***L. monocytogenes*** causes **meningitis** and **sepsis** in newborns, pregnant women, and immunosuppressed adults.

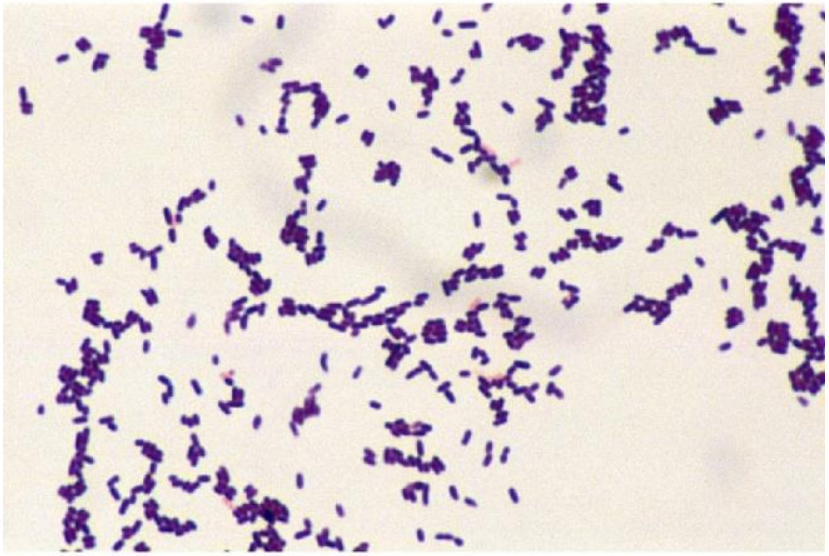
-- It may also cause **febrile gastroenteritis**.

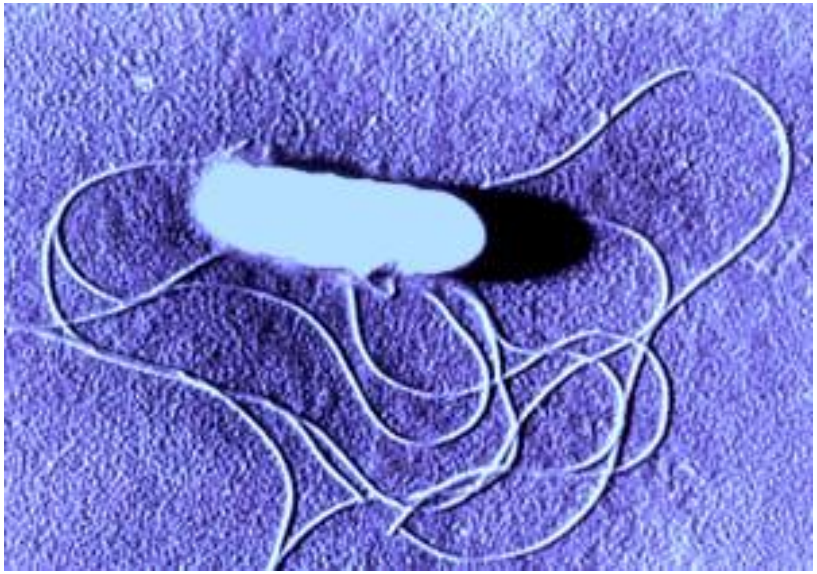
-- It is a major cause of concern for the **food industry**.

Important properties

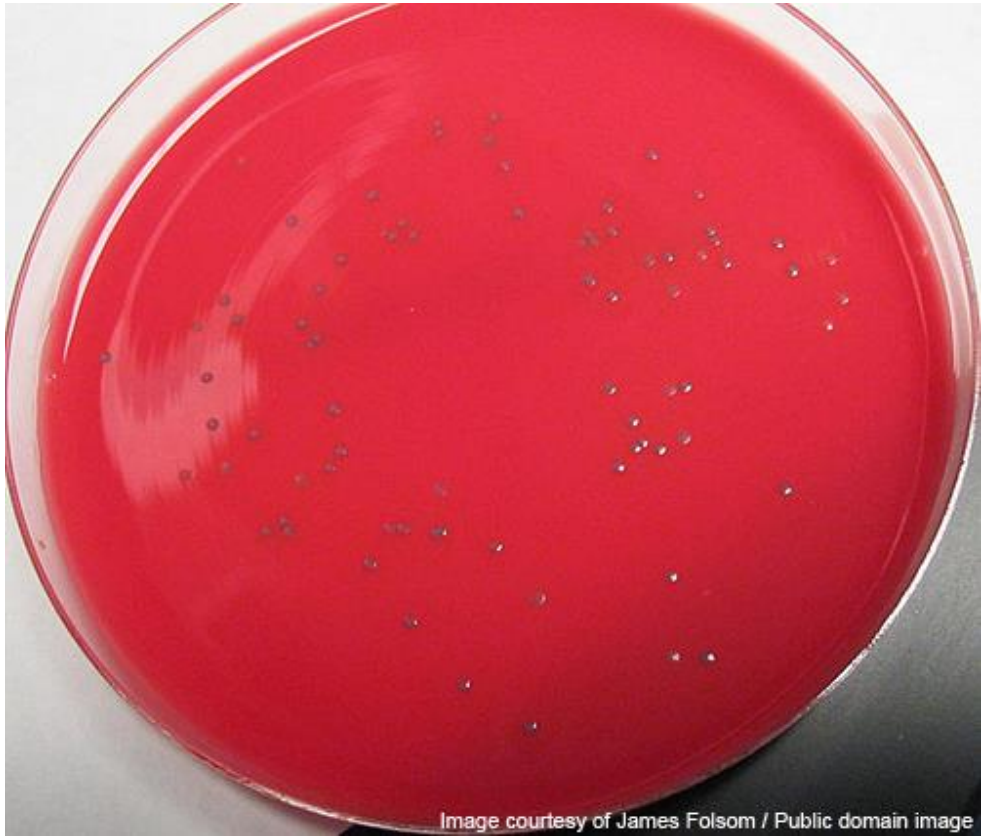
-- These are **small Gram positive rods** arranged in V or L shaped formations similar to corynebacteria.

-- They are different from corynebacteria in being **motile with a characteristic tumbling movement.**





-- Colonies on blood agar produce a **narrow zone of β-hemolysis**.



-- They can grow well at cold temperature so they are a problem in food stored in refrigerators.

Pathogenesis

L. monocytogenes infections have two clinical settings:

1. Transmission across **placenta**
2. In **pregnant women** and **immunosuppressed adults**, esp. **renal transplant** patients.

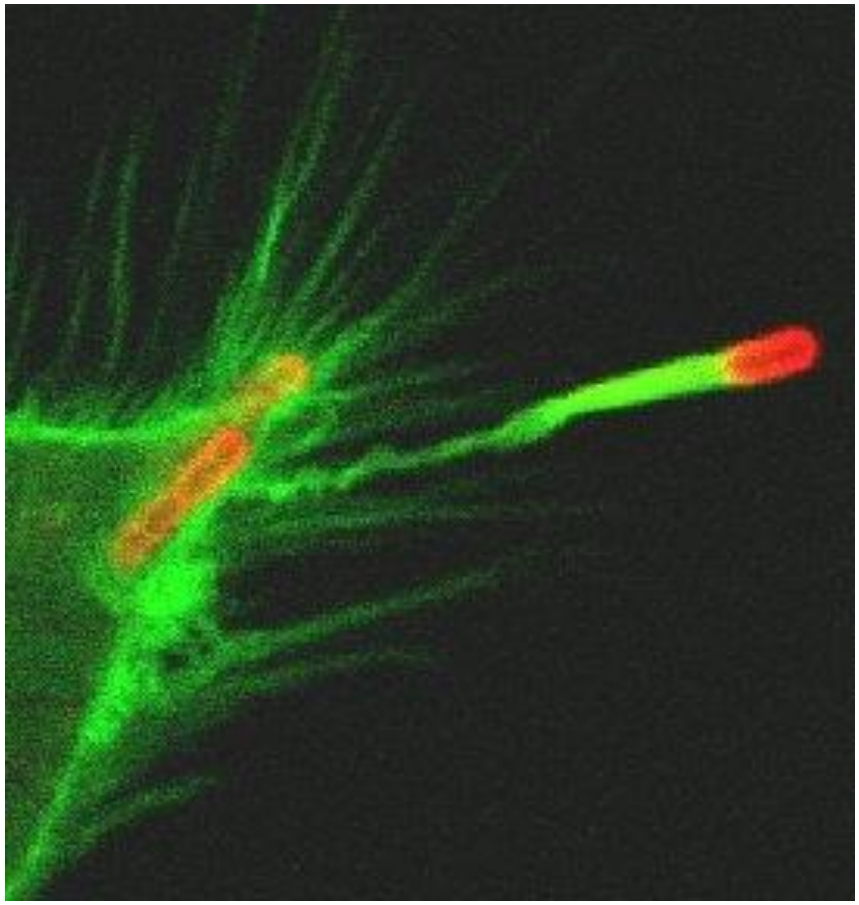
The organism has **worldwide distribution** in animals, soil and plants and is transmitted to human through **animal and plant food products** or **contact.**

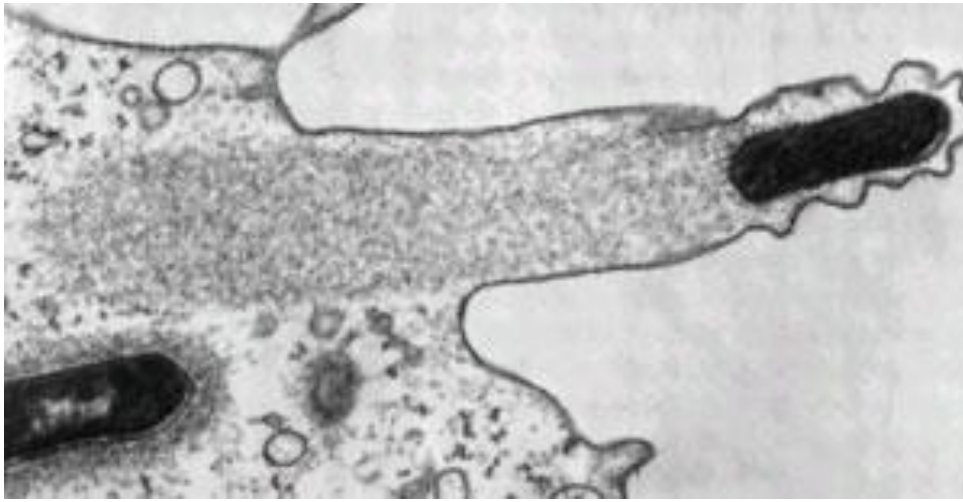
-- Following ingestion, bacteria appear in **colon** and then can colonize the **female genital tract** from where they can **infect the fetus**.

-- They produce a protein **internalin** which interacts with **E-cadherin** on surface of human cells and thus facilitate intracellular survival, passage through placenta and meninges, and invasion of GIT.

-- Due to **intracellular survival**, cell mediated immunity is more important.

They move from cell to cell by **actin rockets**.





Clinical findings

-- Infection during pregnancy can cause **abortion, premature delivery**, or **sepsis** during peripartum period.

-- Infected newborns can have **acute meningitis** 1-4 weeks later.

-- **It reaches meninges through bloodstream**.

-- Infected mother is **asymptomatic** or has **influenza like symptoms**.

-- Infection in **immunocompromised adults** can be either **sepsis** or **meningitis**.

-- **Gastroenteritis** is characterized by watery diarrhea, fever, headaches, myalgias, and abdominal cramps but **little vomiting**.

Laboratory diagnosis

-- It depends primarily on **Gram stain** and **culture**.

-- Diphtheroid like organisms on Gram stain and characteristic colonial morphology on blood agar are diagnostic.

-- **Motility** is differentiating factor.

-- **Sugar fermentation tests** are helpful.

Treatment

-- **Trimethoprim-sulfamethoxazole** is drug of choice for meningitis and sepsis.

-- Combinations with **ampicillin** and **gentamicin** can also be used.

Prevention

-- It is difficult because **no immunization** is available.

-- Limitation of exposure of pregnant women to **animals and their products** can be helpful.

Aerobic Spore forming Gram positive rods

Bacillus

Two medically important species are ***B. anthracis*** and ***B. cereus***.

B. anthracis

Disease

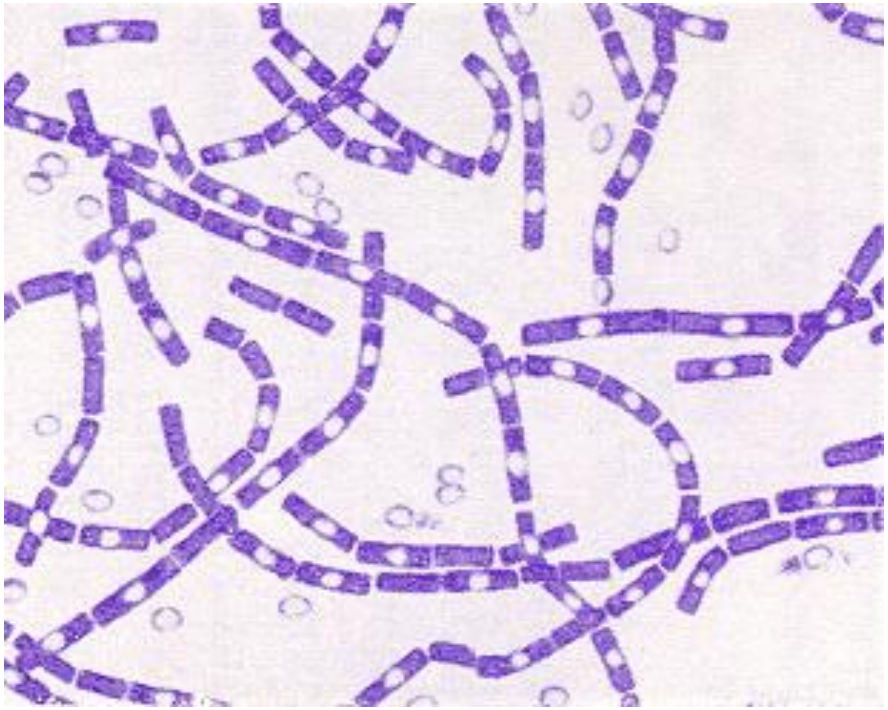
-- They cause **anthrax** which is **common in animals** but **rare in humans**.

-- **Human disease** occurs in three forms: **cutaneous, pulmonary (inhalation)** and **gastrointestinal**.

Important properties

-- These are **large Gram positive rods with square ends**, frequently found in chains.

-- The **spore is central and bulging**.



-- There is a **capsule composed of D-glutamate** (capsules of other species are polysaccharide in nature).

-- These are **non motile** whereas other members of *Bacillus* are motile.

-- **Anthrax toxin** is encoded on **one plasmid**, and **polyglutamate capsule** on a **different plasmid**.

Transmission

-- The **spores** can survive in soil for a very long time and are **transmitted to human from animal products**, such as hides and wool, at the time of **trauma on skin**.

-- The spores can also be **inhaled** to cause **pulmonary anthrax**.

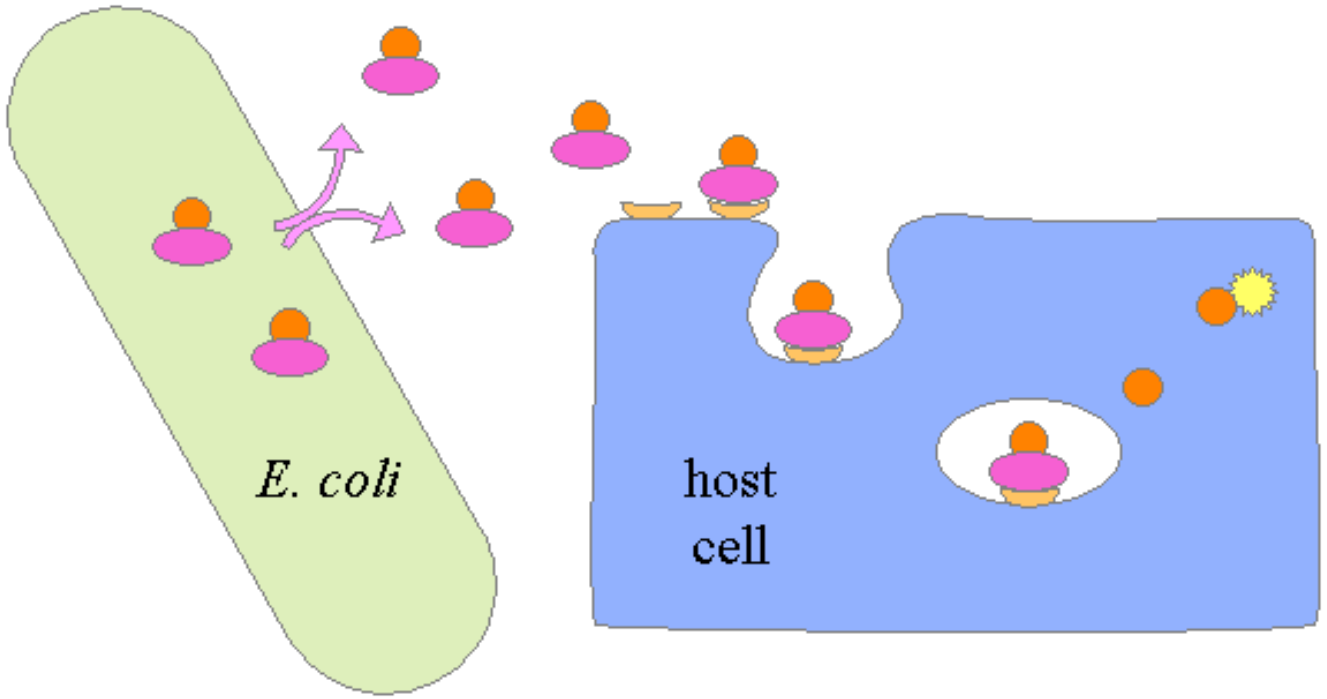
-- **GIT anthrax** is due to ingestion of contaminated meat.

-- **Pulmonary anthrax** is **not communicable** as organisms move rapidly to mediastinal lymph nodes to cause **hemorrhagic mediastinitis**.

Pathogenesis

-- It is based primarily on production of **two exotoxins**, **edema factor** and **lethal factor**.

-- Each has two proteins in **A-B subunit configuration**.



-- **Edema factor** is an **adenylate cyclase**.

-- It causes an increase in the intracellular concentration of cyclic AMP resulting in **outpouring of fluid from the cell** into the extracellular space which appears as edema.

-- **Lethal factor** is a **protease** that cleaves the phosphokinase that activates the **mitogen activated protein kinase (MAPK)** signal transduction pathway. This pathway controls the growth of human cell which is therefore stopped.

-- There is a third factor, the **protective antigen** (against which antibodies are produced) which forms pores in cell membrane to allow the toxins to enter.

Clinical findings

-- Typical lesion of **cutaneous anthrax** is a **painless ulcer** with a black eschar with local edema.

-- The lesion is called **malignant pustule**.

-- Untreated patients progress to **bacteremia** and **death**.

-- **Pulmonary anthrax** or **“wool sorter’s disease”** starts with non specific symptoms resembling influenza.

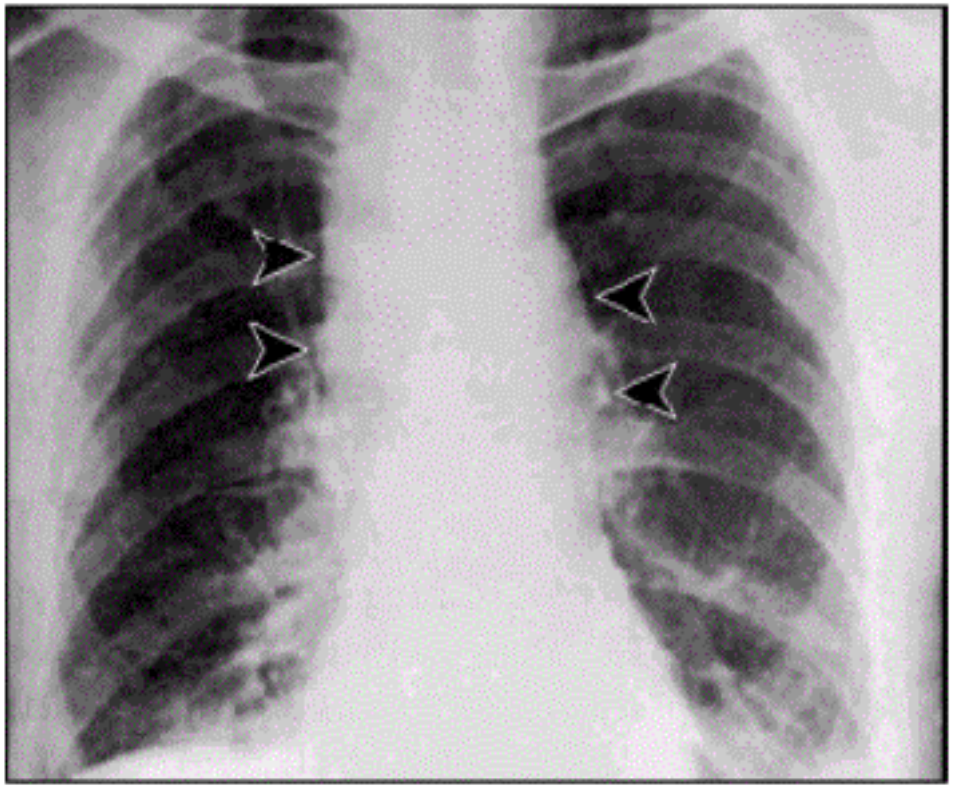
-- It rapidly progresses to **hemorrhagic mediastinitis, bloody plural effusions, septic shock and death**.

-- Lungs are infected but classic**al picture of pneumonia is absent**.

-- Symptoms of **GIT anthrax** include **vomiting, abdominal pain**, and **bloody diarrhea.**

-- **Hemorrhagic mediastinitis** and **hemorrhagic meningitis** are severe life threatening complications.

-- **Mediastinal widening** seen on chest X-ray is important for diagnosis.



Laboratory Diagnosis

-- **Smears** show **large, Gram positive rods in chains**.

-- **Spores are not usually seen** because there is no deficiency of nutrients.

-- **Non hemolytic** colonies form on blood agar aerobically.

-- In **suspected terror attacks**, rapid diagnosis can be made by **PCR** or **direct fluorescent antibody test** that detects antigens in lesions.

-- **ELISA** for antibodies can only be used to make a diagnosis **retrospectively**.

Treatment

-- **Ciprofloxacin** is the drug of choice.

-- **Doxycycline** is an alternative.

-- **No resistant strains** have been isolated.

Prevention

-- People at high risk can be immunized with **cell free vaccine** containing **purified protective antigen** as immunogen.

-- It is **weakly immunogenic** and six doses are given over an 18 month period.

-- Annual boosters are needed.

-- **Incineration of dead animals** instead of burying in soil is recommended.

B. cereus

-- It causes **food poisoning in cooked rice**.

-- Spores survive **steaming** and **rapid frying** and spores germinate if rice are kept warm for many hours.

-- There are **two exotoxins**, one act in the same way as **cholera toxin**, i-e, **ADP-ribosylation**.

-- Mode of action of other toxin is **similar to Staphylococcal exotoxin**.

-- Disease may appear after **short incubation period** (4 hours) with **nausea and vomiting** or after **long incubation period** (18 hours) featuring **watery, non bloody diarrhea**.

-- **Treatment is symptomatic** and only protection is not to keep rice `warm for long period.


Thank You!