# The gram-positive rods

Lec. 6

# CORYNEBACTERIA

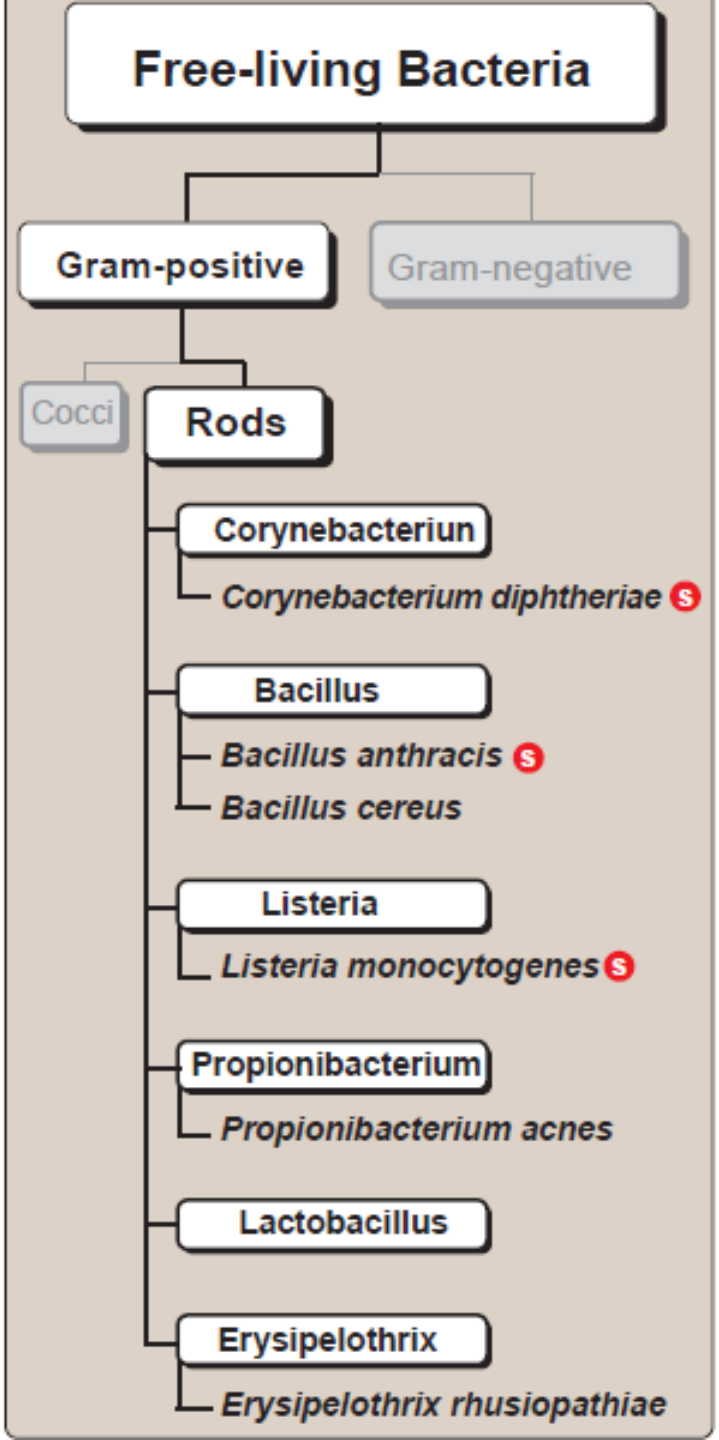
From the Greek koryne---club
Small, pleomorphic
I*ncludes many species of aerobic and facultative anaerobes.*
The cells tend to have clubbed ends, and often remain attached after division, forming “Chinese letter” or palisade arrangements.

Non Spores former. Non motile

Growth is generally best under aerobic conditions on media enriched with blood or other animal products, but many strains will grow anaerobically.

Colonies on blood agar are typically small (1 to 2 mm), and most are nonhemolytic.

Catalase is produced, and many strains form acid (usually lactic acid) through carbohydrate fermentation.



# *Corynebacterium diphtheriae*

*C. diphtheriae produces exotoxin,* Other corynebacteria are called diphtheroids
C. diphtheriae is found in the throat and naso-pharynx 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.
*C. diphtheriae is transmitted by droplet spread, by direct contact with cutaneous infections,* and, to a lesser extent, by fomites.
*C. diphtheriae has little invasive capacity, and diphtheria is due to the local and systemic* effects of DT, a protein exotoxin with potent cytotoxic features.
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.
Inside the cell, fragment A separates from fragment B and catalyzes a reaction between nicotine adenine dinucleotide (NAD+) and the eukaryotic polypeptide chain elongation factor, EF-2.
The toxin is encoded on a β-coryne - phage and only those strains in which the phage is integrated into the *C. diphtheriae* chromosome produce toxin.
Toxin gene expression is also regulated by environmental conditions. Low iron conditions induce toxin expression, whereas high iron conditions repress toxin production.

**Upper respiratory tract infection: Diphtheria is a strictly localized** infection, usually of the throat.

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. Cardiac conduction defects and myocarditis may lead to congestive heart failure and permanent heart damage.

**Cutaneous diphtheria: A puncture wound or cut in the skin can** result in introduction of C. diphtheriae into the subcutaneous tissue, leading to a chronic, nonhealing ulcer with a gray membrane.

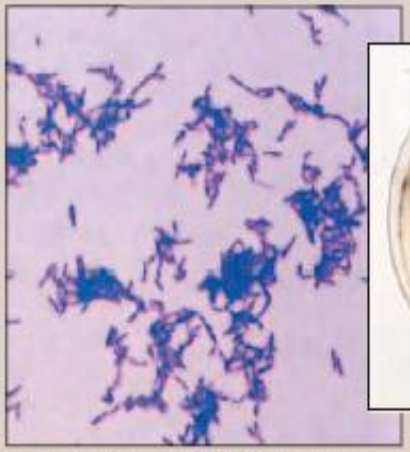
Lab dx:
The initial diagnosis of diphtheria is clinical
Direct smear is unreliable since C. diphtheriae is morphologically similar to other coryneforms.
Definitive diagnosis is accomplished by isolating and identifying *C. diphtheriae from the infected* site and demonstrating its toxigenicity. Isolation is usually achieved with blood agar and culturing on a selective medium containing potassium tellurite (eg, Tinsdale medium).
Toxigenicity test (production of DT) by agar immunopreciptation test (Elek test*) or by tissue culture cytotoxicity assay.
Toxin gene can be detected by PCR and guinea pig.
Measuring Abs to diphtheria toxin in serum collected before administration of antitoxin.
Schick test: An intradermal injection used to determine individual susceptibility to the toxin. A localized erythema and sometimes sever local reaction reaching maximum size and intensity in 2-4 days indicate that there is little or no neutralizing antitoxin in the tissue and the individual is susceptible to diphtheria, abscence of reaction indicate immunity.
*Elek test: culture are streaked horizantally, then overlayed by an antitoxin impregnated strip. Toxin and antitoxin diffuse into culture During incubation and preciptin lines develop where toxin and antitoxin are present.

Rx:
Antitoxin (hyperimmune horse serum) is given
Pencillin or oral erythromycin.

Vaccine:
Toxoid

Gram (+) rods

# *Corynebacteria* species



*Corynebacterium diphtheriae*
Gram stain

*Corynebacterium diphtheriae* grown on tellurite blood medium

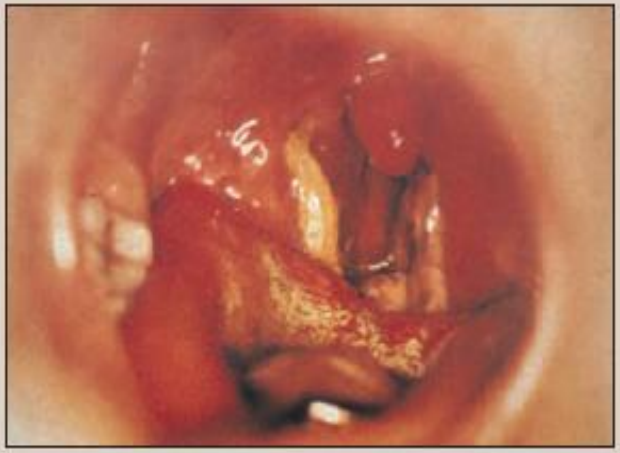
- **Small, slender, pleomorphic rods form characteristic clumps that look like Chinese characters or a picket fence.**
- **They are nonmotile and unencapsulated.**
- **Most species are facultative anaerobes.**
- **Culture aerobically on selective medium, such as Tinsdale agar containing tellurite (an inhibitor of the other respiratory flora).**

## *Corynebacterium diphtheriae*

- Diphtheria[1]

1. Erythromycin
2. Penicillin G

[1]Treatment of diphtheria requires prompt neutralization of toxin, followed by eradication of the organism. A single dose of horse serum antitoxin inactivates any circulating toxin, although it does not affect toxin already bound to a cell-surface receptor.



*Corynebacterium diphtheriae* infection of the throat. Gross swelling and congestion of the whole pharyngeal and tonsillar area, with a gray exudate covering the tonsil.

*Listeria monocytogenes*
*Gram-positive rod with some bacteriologic features that resemble* those of both corynebacteria and streptococci.

In stained smears of clinical and laboratory material, the organisms resemble diphtheroids.
*Listeria are not difficult to* grow in culture, producing small, β-hemolytic colonies on blood agar.
*Listeria species are catalase* positive, which distinguishes them from streptococci, and produce a characteristic tumbling motility in fluid media at 25°C that distinguishes them from corynebacteria.

This species is able to grow slowly in the cold even at temperatures as low as 1°C

Eleven *L. monocytogenes serotypes are recognized based on flagellar and somatic surface* antigens, but the majority of human cases are limited to only three serotypes (1/2a, 1/2b, 4b). These serotypes differ from other *Listeria in elements of the chemical teichoic* acid composition, a major component of their cell wall.

**EPIDEMIOLOGY**

Members of *Listeria are widespread among animals in nature, including those associated* with our food supply (eg, fowl, ungulates). The human reservoir appears to be intestinal colonization, which various studies have shown to range from 2 to 12%.

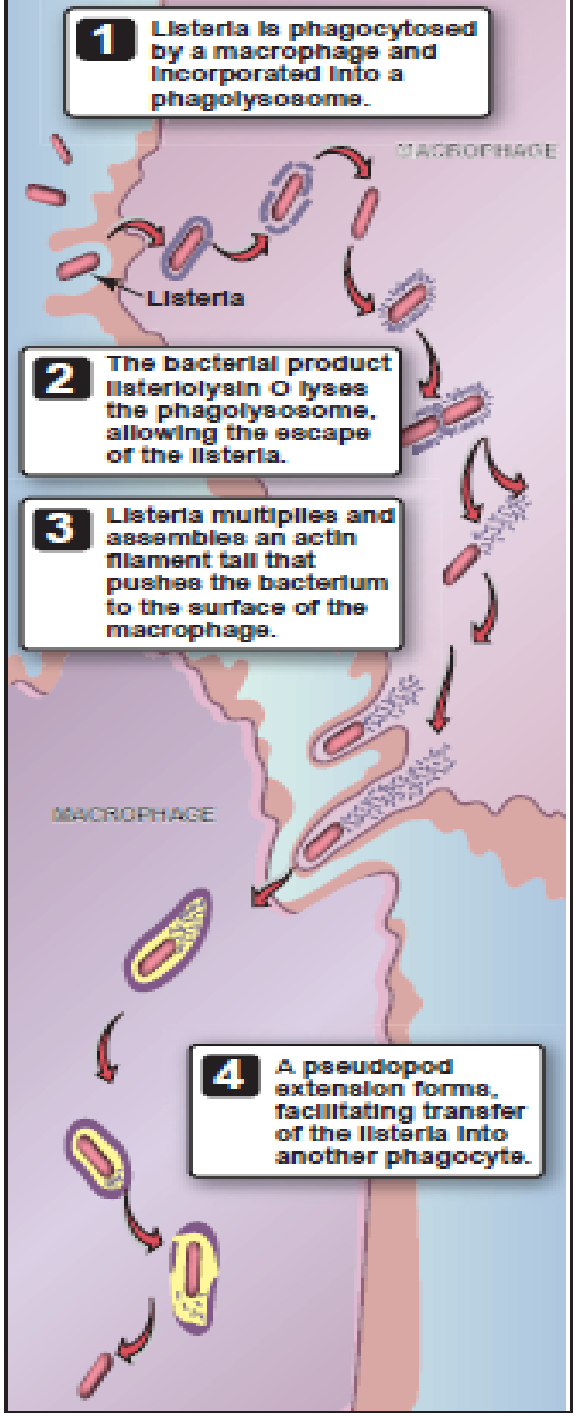
Most of the cases were among mother–infant pairs.

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. monocytogenes to grow at refrigerator temperatures,* allowing scant numbers to reach an infectious dose during storage. Heightened awareness has implicated many other foodstuffs, particularly those prepared from animal products in a ready-to-eat form such as sausages.

*L. monocytogenes may 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.

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

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.



Once in the cytosol, *L. monocytogenes continues to move through the cell by controlling* the metabolism of the cell's actin filaments. This process is stimulated by other surface proteins (ActA, gelsolin), which control the actin polymerization so that actin monomers are sequentially concentrated directly behind the bacterium. The net effect is the appearance of a bacterial "tail" that is connected to the long actin filaments. The addition of new actin units to the tail propels the organisms through the cytosol like a comet through the evening sky. The motile *Listeria eventually reach the edge of the* cell where, instead of stopping, they protrude into the adjacent cell taking the original cell membrane along with them. When these pinch off, the organisms are surrounded by a double set of host cell membranes that are dissolved by LLO and phospholipases, releasing the organisms to start the cycle again.

**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 Utero transmission, sepsis with high mortality, disseminated granulomas with central necrosis.
Late onset: 2-3 weeks after birth from fecal exposure, meningitis with septicemia.

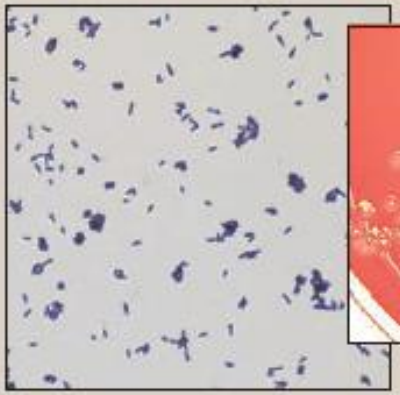
Immunocompromised patients: septicemia and meningitis.

**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.

Gram (+) rods

# *Listeria* species



*Listeria monocytogenes* in cerebrospinal fluid (Gram stain)

*Listeria monocytogenes* on blood agar

- Slender, short rods, sometimes occurring as diplobacilli or in short chains
- Intracellular parasites
- Catalase positive
- Distinctive tumbling motility in liquid medium
- Grow facultatively on various enriched media

***Listeria monocytogenes***

- Listeriosis

1. Ampicillin
2. Trimethoprim/ sulfamethoxazole

# Bacillus and Clostridia

G+ve rods

*B anthracis* -----Anthrax

*B cereus*------- Food poisoning

*B. anthracis* has been classified by the CDC as a Category A bioterrorism agent due to its high lethality, and ease of weaponization.

Spore is oval, central in position

Tem range of growth 12—45 °c ( optimum 35 °c)

Grows on all ordinary media as typical colonies with wavy margin and small projections, the so called medusa head appearance .

The spores are resistant to chemical disinfectants and heat: the spores of many strains will resist dry heat at 140°c for 1-3h and boiling or steam at 100c for 5-10 min.

Autoclaving destroys them in 15 min.

**Virulence factors**

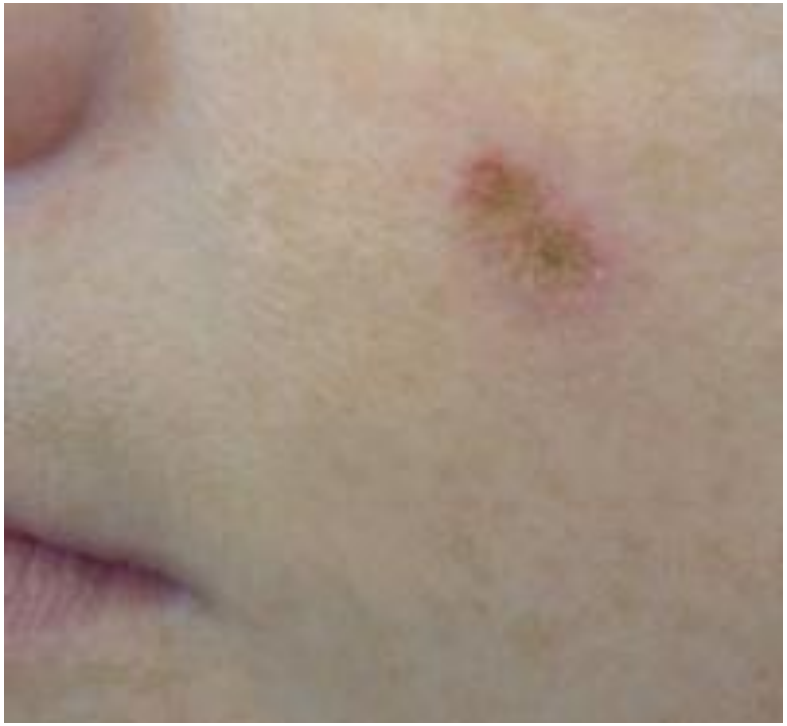
a. Antiphagocytic capsule

b. Plasmid-encoded toxin complex comprising three proteins, all of which are required for pathogenicity: the protective antigen, oedema factor and lethal factor)

Diseases:

Man is relatively resistant to infection.

Inoculation through skin of material from infected animals or their products----cutaneous anthrax (malignant pustule)



**Respiratory Anthrax (woolsorters' disease )**
Inhalation of spores in dust or wool fibers

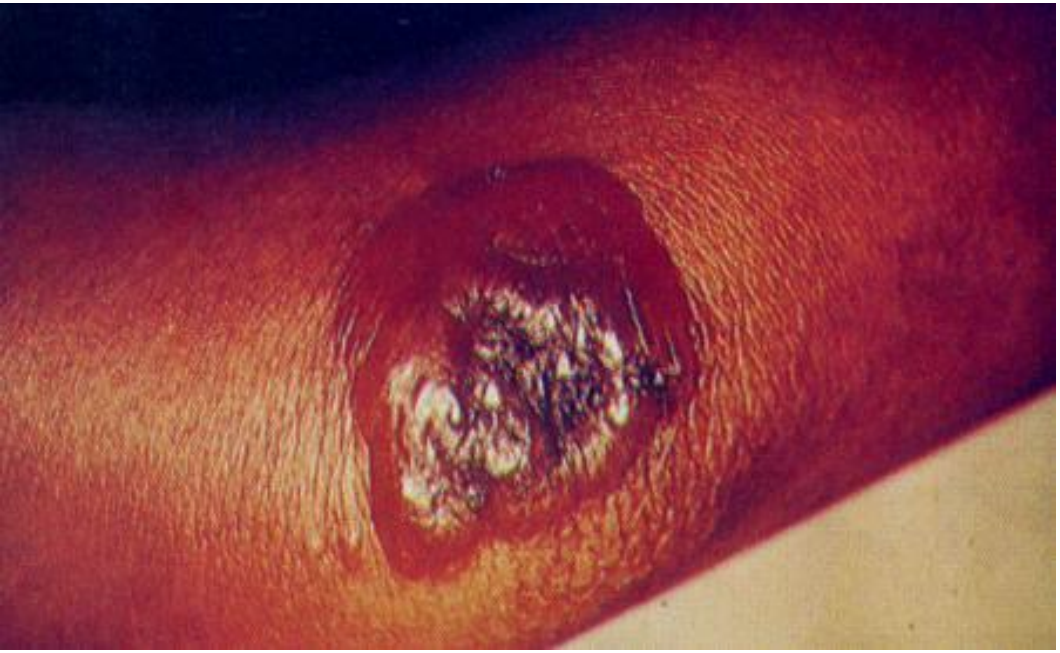
**Intestinal Anthrax**
It occurs from eating infected, undercooked meat.

# Anthrax

## Pathogenesis and clinical presentations

### Cutaneous anthrax

About 20% mortality



**Virulence factors**

**Capsule**

**(antiphagocytic)**

**Toxin**

**(oedema & death)**

### Inhalation anthrax

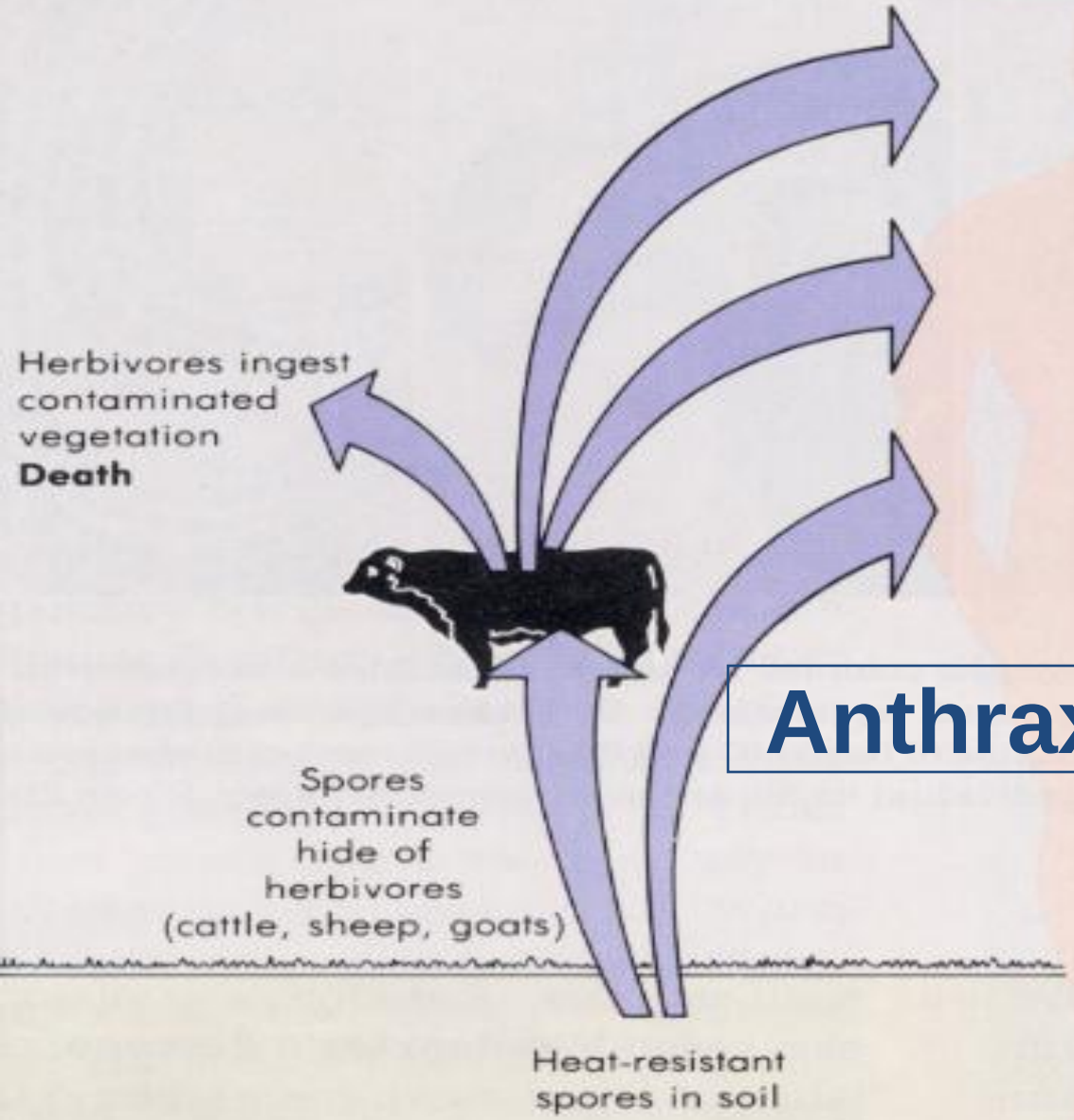
High mortality

### Gastrointestinal anthrax

High mortality

# Anthrax - Epidemiology

| Primary hosts | Accidental host | |
|---|---|---|
| | Method of infection | Mortality |
| | *Contact with contaminated animal products (hides, wool, hair) | 20% |
| | Ingestion of contaminated food products | 95% |
| | Inhalation of contaminated dust (Woolsorter's disease) | 95% |




*Contact infections are generally on exposed skin surfaces (e.g., arms) and result in 95% of infections

**Laboratory Identification – Lab safety is critical when suspecting *B. anthracis***

a. Gram stain – large, square-ended, gram-positive or gram-variable rods in singles or chains

 Central spores are generally not present in clinical samples (spores are never found in the tissues, but appear when the organism is shed or grown on artificial media).

 Presence of capsule (clear zones around cells) strongly presumptive ID

b. Colony morphology – non-hemolytic, large, gray flat colonies with irregular margins (filamentous projections – Medusa head)

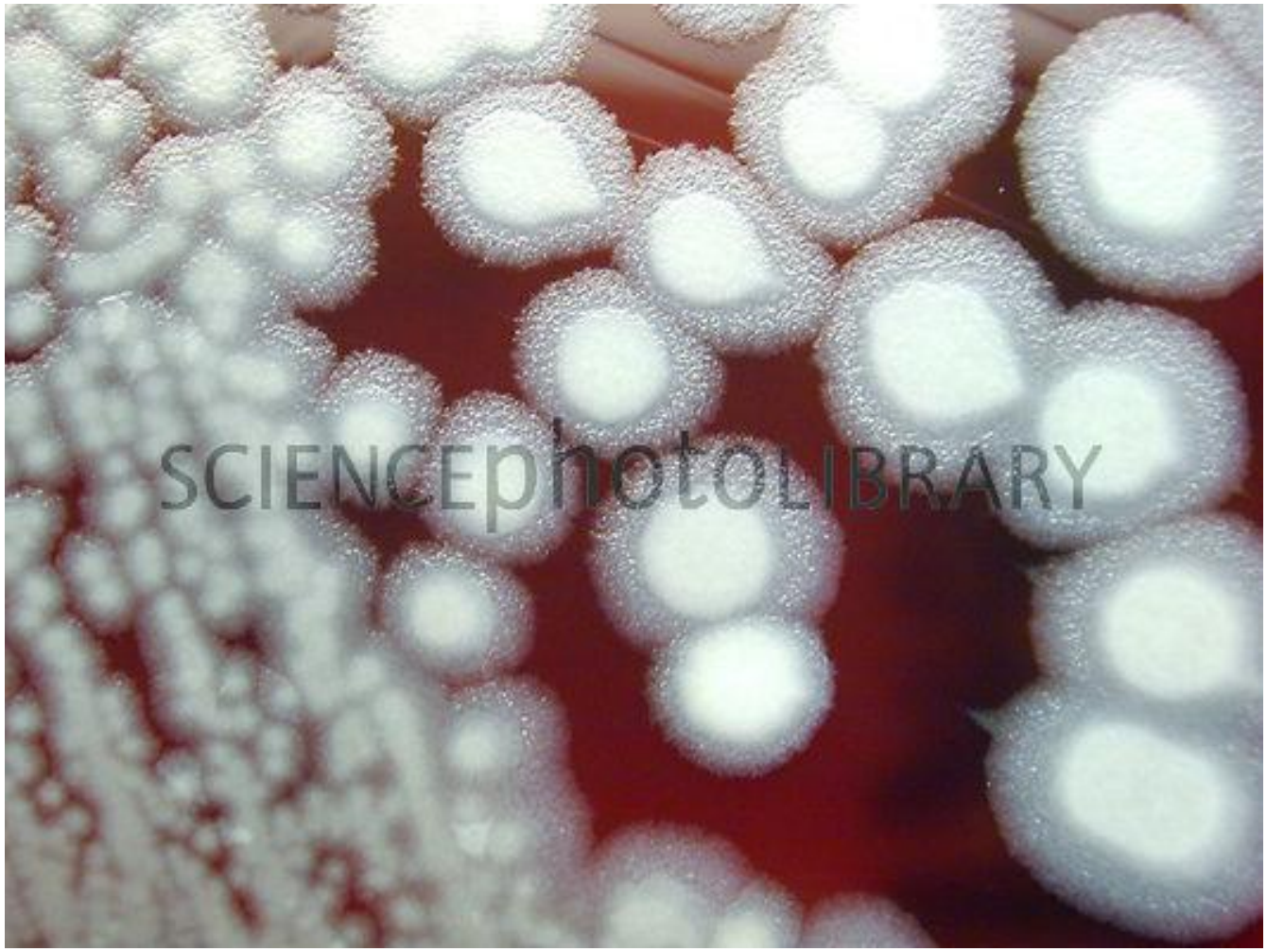
Motility: -ve

Gelatin liquefaction

Serological test (ELISA)

Penicillin – Sensitive

 **Suspected colonies should be sent to reference laboratory**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# Anthrax – treatment and prevention

- **Penicillin**
- **(Tetracycline /chloramphenicol)**
- Erythromycine,Clindamicine
- **Prevention**
  - **Vaccination of animal herds**
  - **Proper disposal of carcasses**
- **Active immunisation with live attenuated bacilli**

*B. Cereus*

*Large gram +ve (motile, lacks the capsule)*

*Resemble B anthracis in culture forming large, grey, irregular colonies*

**Clinical Significance**

a. Food poisoning – food contaminated with organism or toxins formed by organism

 Diarrheal type – abdominal pain and watery diarrhea caused by enterotoxin

o Associated with poultry, meats, soups, vegetables and desserts, symptoms usually 8- 16 hours after ingestion, recover 12-24 hours from onset

 Emetic type – vomiting caused by emetic toxin

o Associated with fried or boiled rice, symptoms usually 1-5 hours after ingestion, recover 6-24 hours after onset

 Both diarrheal and emetic forms are usually mild and self-limiting

b. Serious infections in immunocompromised host

**Laboratory Identification**

a. ***B. cereus is normal stool flora, to diagnose food poisoning must culture suspected*** **food NOT stool**

b. Gram stain – large, gram-positive rods with spores, can stain gram-variable or gram-negative

c. Colony morphology – beta-hemolytic; large, feathery, spreading on Agar

d. Preliminary Identification

- Beta-hemolytic
- Motile
- Penicillin – Resistant

Clostridium spp.
Most Clostridium species decompose proteins or form toxins and some do both.
Their natural habitat is the soil or intestinal tract as saprophytes.

The important pathogenic species are:
- Clostridium botulinum: Causes botulism
- Clostridium tetani: Causes tetanus
- Clostridium perfringens: Causes gas gangrene
- Clostridium difficile; causes pseudomembranous colitis and antibiotic-associated diarrhoea.

Note: the toxins produced by the organisms of tetanus and botulism attack nervous pathways (neurotoxins), the organisms associated with gas gangren attack soft tissues by producing toxins (histotoxic). C difficile and some strains of c. perfringens produce an enterotoxin

Morphology
- Large anaerobic gram positive motile rods.
- The spore is usually wider than the rods.
- Spores are placed centrally, terminally, or subterminally according to the genus.

Unable to utilize O2 as the final oxygen acceptor.
Oxidase, catalase -ve

Types of Clostridium botulinum: They are from A – H according to the type of toxin produced.

 Types A, B, and E are the most commonly associated with illness

 Toxins of types A, B, and E have the following characteristics:

1. They are among the most highly toxic substances known.
2. They are neurotoxic proteins (MW = 150 000)
3. Lethal dose for human is 1 – 2 mg
4. They are destroyed by heating for 20 minutes at 100 oC
5. Toxin production is under the control of a viral gene (Bacteriophage yielded from toxigenic strain and it may infect non-toxigenic strain and convert it to toxigenic).

Action of botulism toxin …

It is a neurotoxic protein. All of its types (A, B, and E) are made of heavy and light chains linked by disulfide bonds. The heavy chain is thought to bind the toxin to the motor nerve end plate. The light chain blocks the calcium-mediated release of acetyl choline. The toxin acts by blocking the release of acetyl choline at synapses and neuromuscular junctions causing flaccid paralysis.

…

Pathogenesis …

Botulism is intoxication. It results from ingestion of food in which Clostridium botulinum spores germinate and produce toxins under anaerobic conditions. These foods are spiced, smoked, vacuum-packed, or canned alkaline foods. The toxin acts by blocking the release of acetyl choline at synapses and neuromuscular junctions causing flaccid paralysis.

Infant botulism …

It may result from honey feeding and cause signs of paralysis or sudden death.

Lab diagnosis …

**Isolation of organism in food/faeces**

**Detection of toxin in faeces / serum**

Toxin is tested by hemoagglutination or radioimmunoassay (RIS).

Treatment …

IV administration of antitoxin (trivalent antitoxin of types A, B, and E).

***Clostridium perfringens***

This species implicated in gas gangrene and certain types of food poisoning.

Capsulated and non-motile

*S*ubdivided into 5 types based on the four major lethal toxins they produce. Type A causes most of the human infections.

Identified by ***Nagler reaction***, which exploits the action of its phospholipase on egg yolk medium; colonies are surrounded by zones of turbidity and the effect is specifically inhibited if *C. perfringens* antiserum containing α-antitoxin is present on the medium.

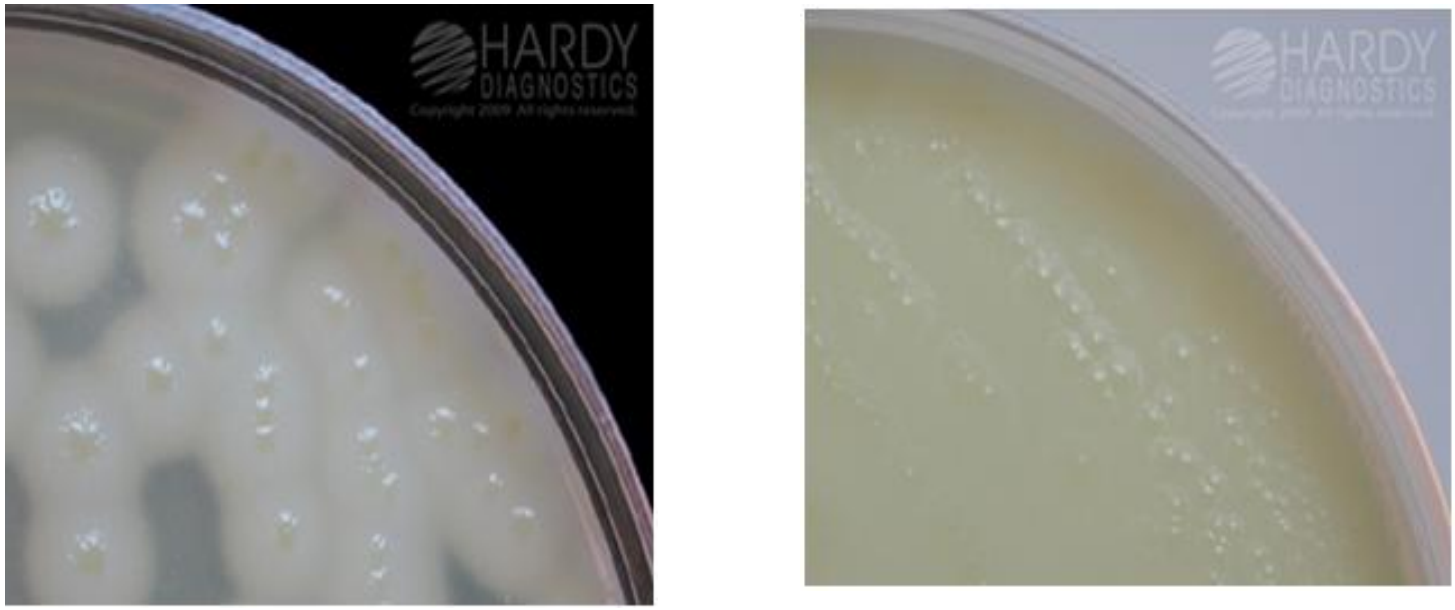
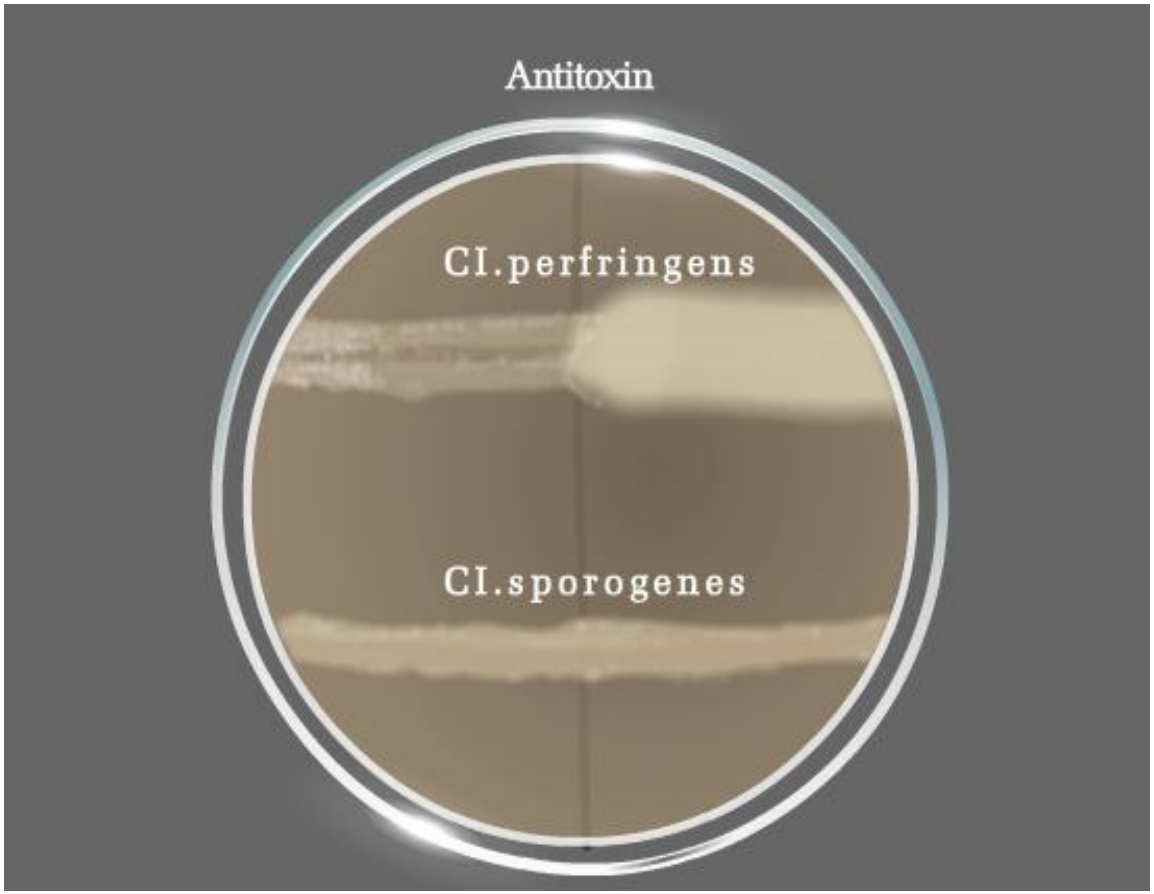



Fig*: Clostridium perfringens* (left) and *Clostridium sordellii* (right) growing on AnaeroGRO™ Egg Yolk Agar


Antitoxin
CI.perfringens
CI.sporogenes

Typical food poisoning strains produce heat resistant spores that can survive boiling for several hours, whereas the spores of strains that cause gas gangrene are inactivated within a few minutes by boiling.

Gas Gangrene: characterized by rapidly spreading oedema, myositis, necrosis of tissues, gas production and profound toxaemia occurring as complication of wound infection.

The main source of organism is animal and human excreta, and spores are distributed widely (soil and even air). The skin often bears spores, especially, in areas of the body that may be contaminated with intestinal organisms.

Pathogenesis:
Impairment of the normal blood supply of tissues with a consequent reduction in oxygen tension may allow an anaerobic focus to develop.
The organisms multiply rapidly and produce a range of toxins (these will damage tissue). Then, they spread into adjacent viable tissue, particularly, muscle, kill it and render it anaerobic and vulnerable to further colonization with the production of more toxin and aggressins.
Hyaluronidase produced by c. perfringens breaks down intercellular cement substance and promotes the spread of the infection along tissue.
Collagenase (liquefy muscle)
Toxins: Alpha toxin, a phospholipase C (lecithinase). Theta toxin: It has hemolytic and necrotic effect on tissue.

# C. perfringens

## Laboratory Diagnosis

Specimens: pus, necrotic tissue, feces, food, etc.

Smears: large gram-positive rods with or without spores, usually in the absence of leukocytes.

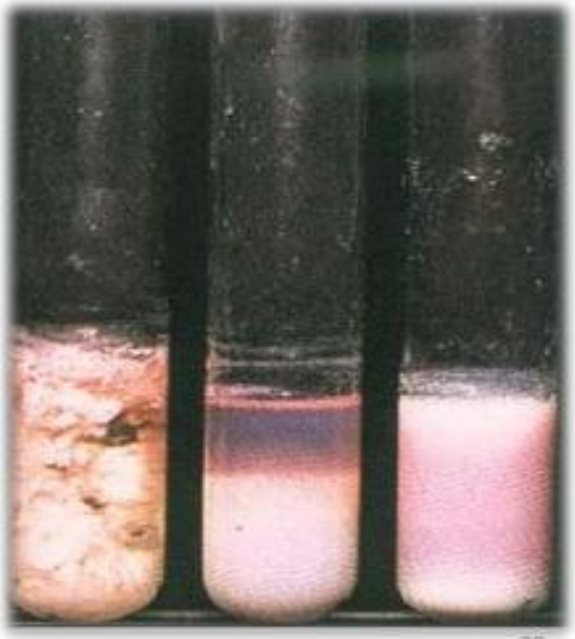
Culture: anaerobic culture on blood plate.

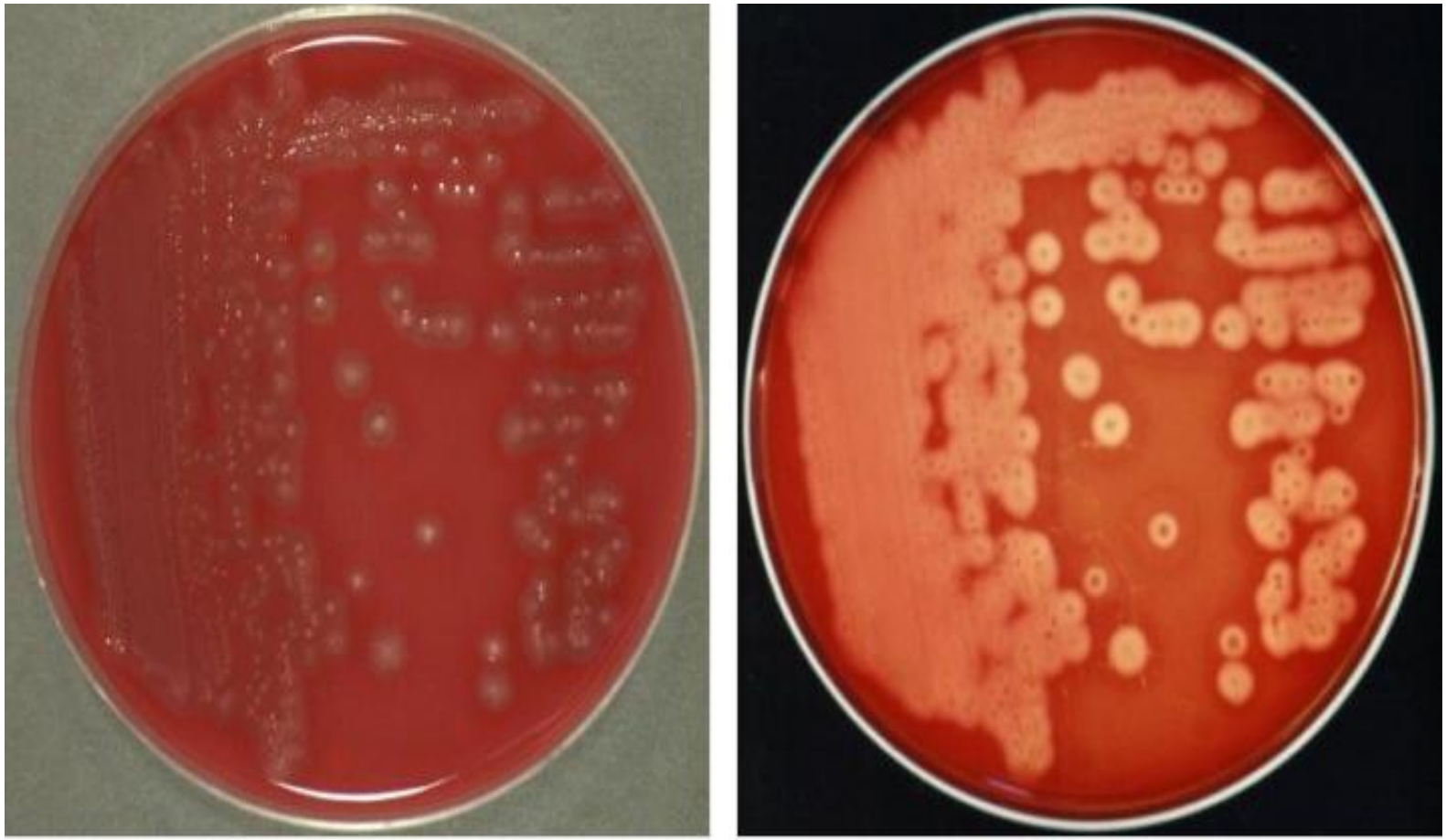
Identification:

"Storming fermentation"-- clot torn by gas in 24 hrs.

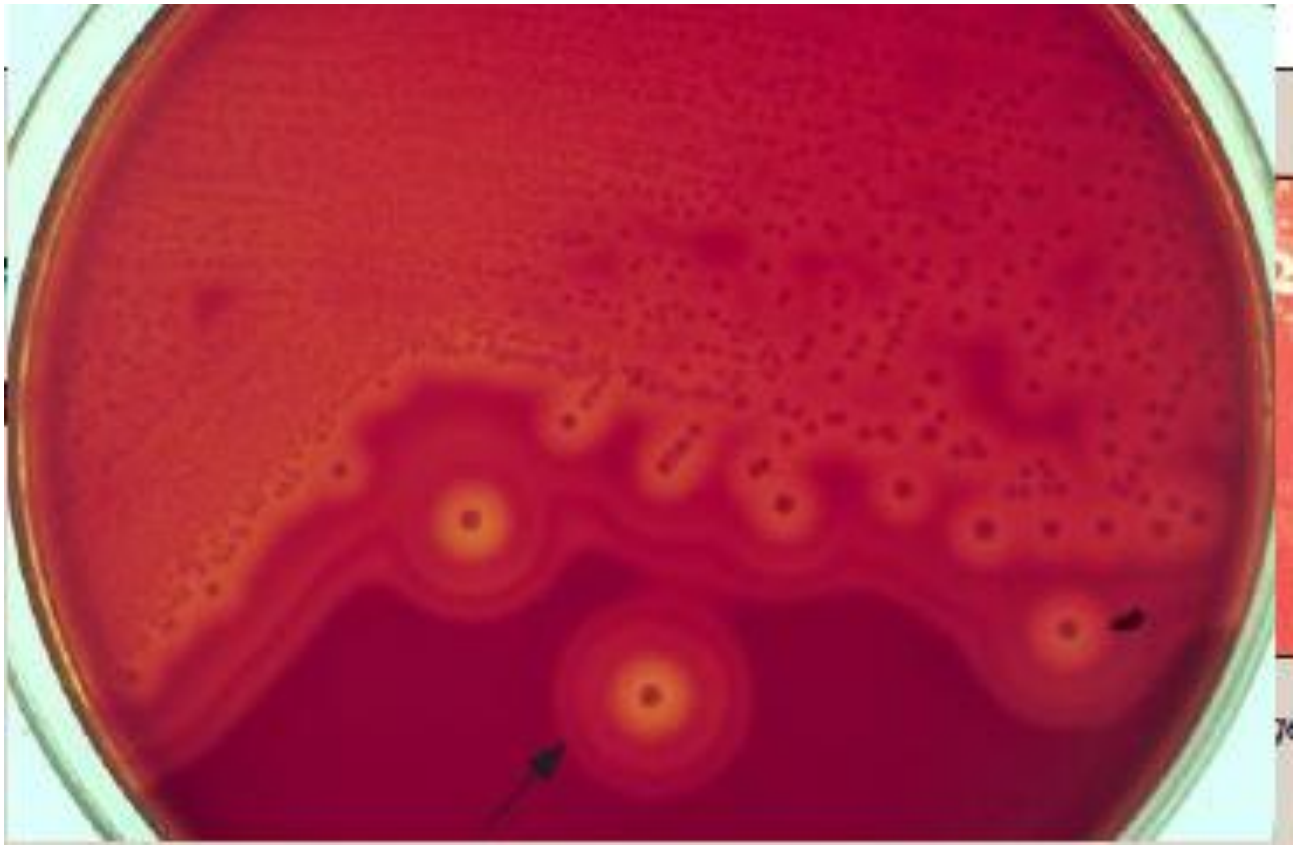
Lecithinase test-- precipitate formed around colonies on egg yolk media.

Biochemical tests.





The same Blood Agar plate examined with transmitted light. Colonies are surrounded by a double-zone haemolysis, which consists of an inner clear zone and an outer hazy zone.



*C. perfringens*

Treatment:
Clinical (surgical)
Antibiotic (immediately in high doses)—mixed infection, co-existence of coliform, gram positive cocci, and fecal anaerobes. Thus Penicillin, metronidazole and an aminoglycoside given in combination.

Food poisining
*C. perfringens* can cause food poisoning if large numbers of an enterotoxin-producing strain are ingested. The spores of some C. perfringens strains are often particularly heat resistant and can withstand temperatures of 100°C for an hour or more. Thus, spores that survive initial cooking can convert to the vegetative form and multiply if food is not refrigerated or is rewarmed. After ingestion, the enterotoxin is released into the upper gastrointestinal tract, causing a fluid outpouring in which the ileum is most severely involved.
*C. perfringens* is the $4^{th}$ most common cause of food poisoning after *campylobacter, salmonella, and staphylococcus.*

***Clostridium tetani***

General characteristics:

- Worldwide in distribution in the soil and in animal feces
- Longer and thinner gram-positive rods with round terminal spores giving characteristic "drum-stick" appearance.
- There are ten antigenic types of c. tetani but all produce the same neurotoxin.
- The toxin has two components:

1. Tetanospasmin: Neurotoxic property
2. Tetanolysin: Hemolytic property

Pathogenesis:

*Clostridium tetani* is not an invasive organism. The infection remains strictly localized in the area of dead tissue (into which the spores have been introduced). Germination of spores to vegetative organisms that produce toxin Tetanospasmin will reach the CNS via the blood and result in generalized muscular spasm.

…Clinical findings of tetanus …

Duration is 4 to 5 days – many weeks. There is muscular contraction of the voluntary muscles ($1^{st}$ area of infection) then the muscles of the jaw (Lock-Jaw disease). Later, other voluntary muscles are involved causing generalized spasm resulting in respiratory paralysis and cardiac failure which lead to death (50%).

 Tetanus neonatrum: Follows contamination of the umbilical cord of newborns when it is cut by contaminated food.

Laboratory diagnosis: The bacteria can be cultured in a media with anaerobic atmosphere. Proof of isolation of C.tetani must rest on production of toxin and its neutralization by specific antitoxin. Diagnosis is exclusively by clinical picture and history of injury.

Prevention …
Active immunization with toxoid (detoxified toxin) to stimulate Ab.
Proper care of wound (Remove the necrotic tissue)
Prophylactic use of antitoxin.
Administration of penicillin (to inhibit Clostridium and pyogenic bacteria)
 Treatment with antitoxin in tetanus neonatrum is life saving.
…Control …
Active immunization of children with tetanus toxoid 3 injections:
 In the 1st year
 Booster injection at entry to school
 Boosters are spaced 7-10 years
Usually in young children: In immunization, tetanus toxoid is combined with diphtheria toxoid and Pertussis vaccine (DTP )

C. difficile
Motile, g +ve , oval subterminal spores.
It occurs commonly in faeces of neonates and babies.
It produce an enterotoxin (toxin A) and a cytotoxin (toxin B).
It is a proven cause of **antibiotic associated diarrhea**, leading to life-threatening condition, pseudomembranous colitis. There is history of antibiotic use (clindamycin, cephalosporins)

Diagnosis:
Sample-faeces (enrichment and selective procedure)
Toxin detection in feces (ELISA)
Treatment:
Discontinuous of Antibiotic Suppress the growth and toxin production by giving oral metronidazole or vancomycin.