Lesson 11

Ethnic microscopic diagnostics of infections, gram-positive chopsticks (Bacillus, Clostridium Corynebacterium, Listeria)

Class Bacillus

- Bacillus anthracis
- Bacillus cereus
- Bacillus subtilis

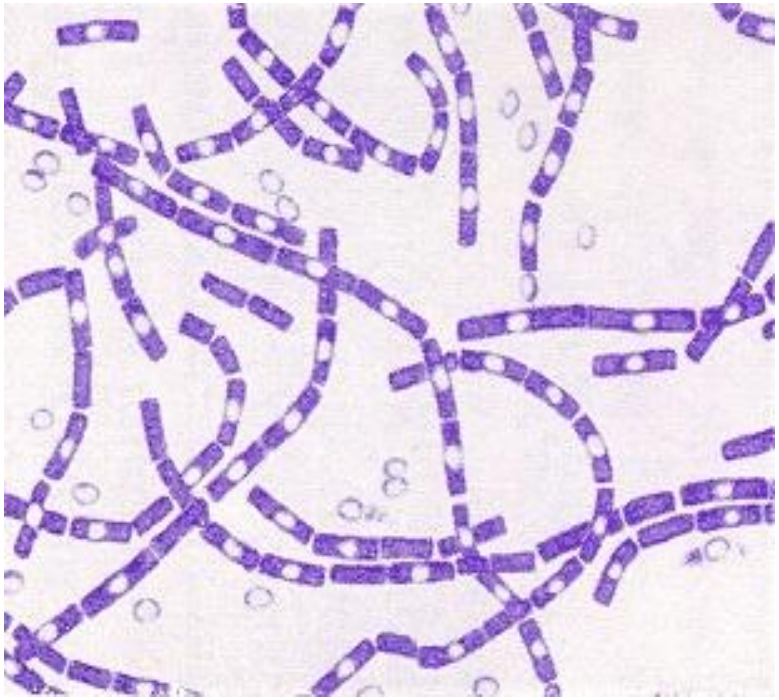
Morpho-biological properties:

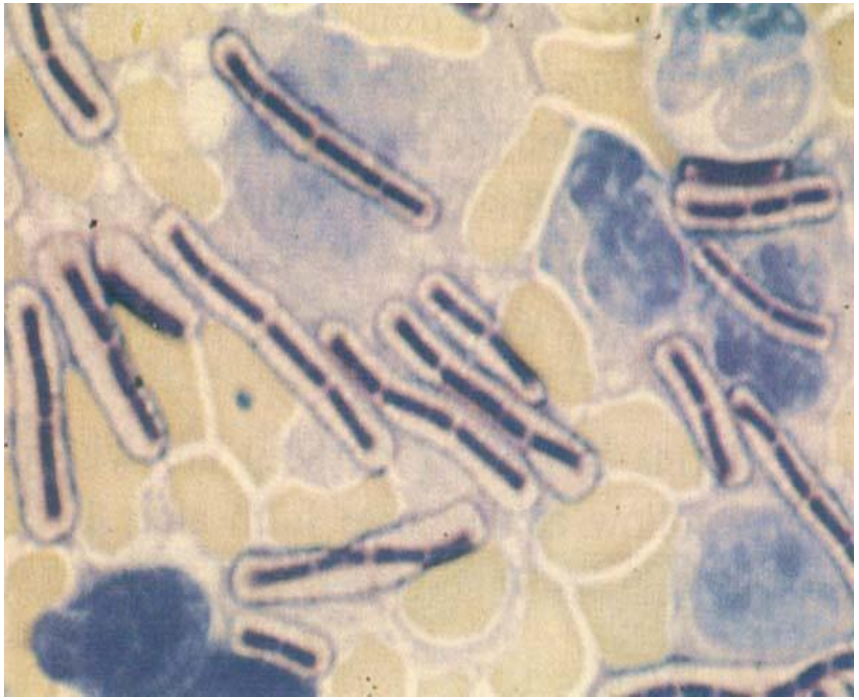
B.anthracis - very large immobile gram-positive rods with chopped ends 1x3-4 microns in size, arranged in a smear in the form of long chains (streptobacilli). They form centrally located spores.

Class Bacillus

- *Belong to the genus Bacillus of the family Bacillaceae*
- *The genus Bacillus includes 48 species. Some species, including B.cereus and B.subtilis, are saprophytes, widespread in nature, and are characterized by mobility.*
- *B.cereus, as a typical representative of the genus Bacillus, when ingested into food, form an enterotoxin and cause food poisoning.*
- *Some species cause opportunistic infections in immunocompromised individuals.*
- *The causative agent of anthrax is Bacillus anthracis, a pathogenic species for humans.*

Bacillus anthracis – the causative agent of anthrax

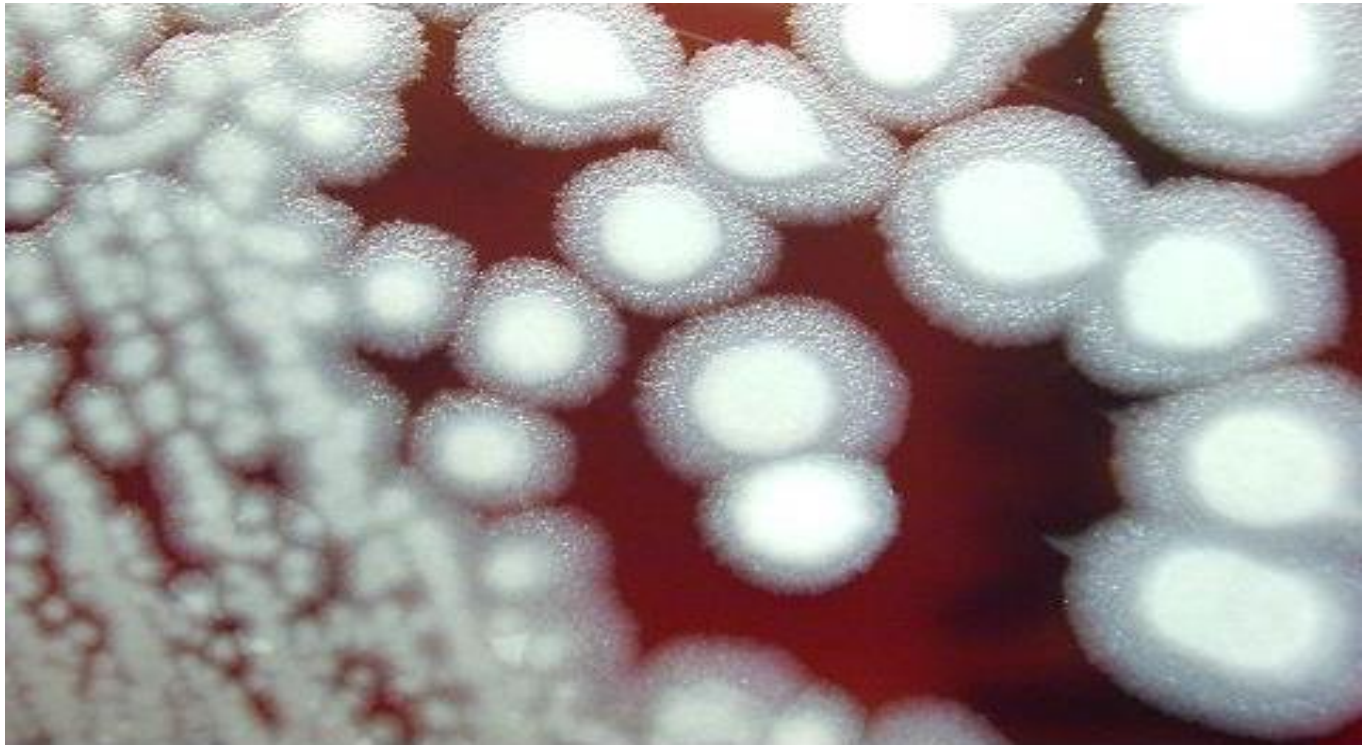




in a Gram-stained smear

Cultural properties:

Aerobe, or facultative anaerobe. Grows well on normal nutrient media. On solid nutrient media forms rough R-colonies with jagged edges. Under a magnifying glass, the colonies resemble a "lion's mane" or "jellyfish's head."



(colonies on blood agar)

Differentiation of bacteria of the genus Bacillus:

Sign	B.anthracis	B.cereus	B.subtilis	B.mesentericus	B.megaterium
Capsule	+	-	-	-	-
Mobility	-	+	+	+	+
Hemolysis	-	+	-	±	-
Pathogenicity	+	-	-	-	-

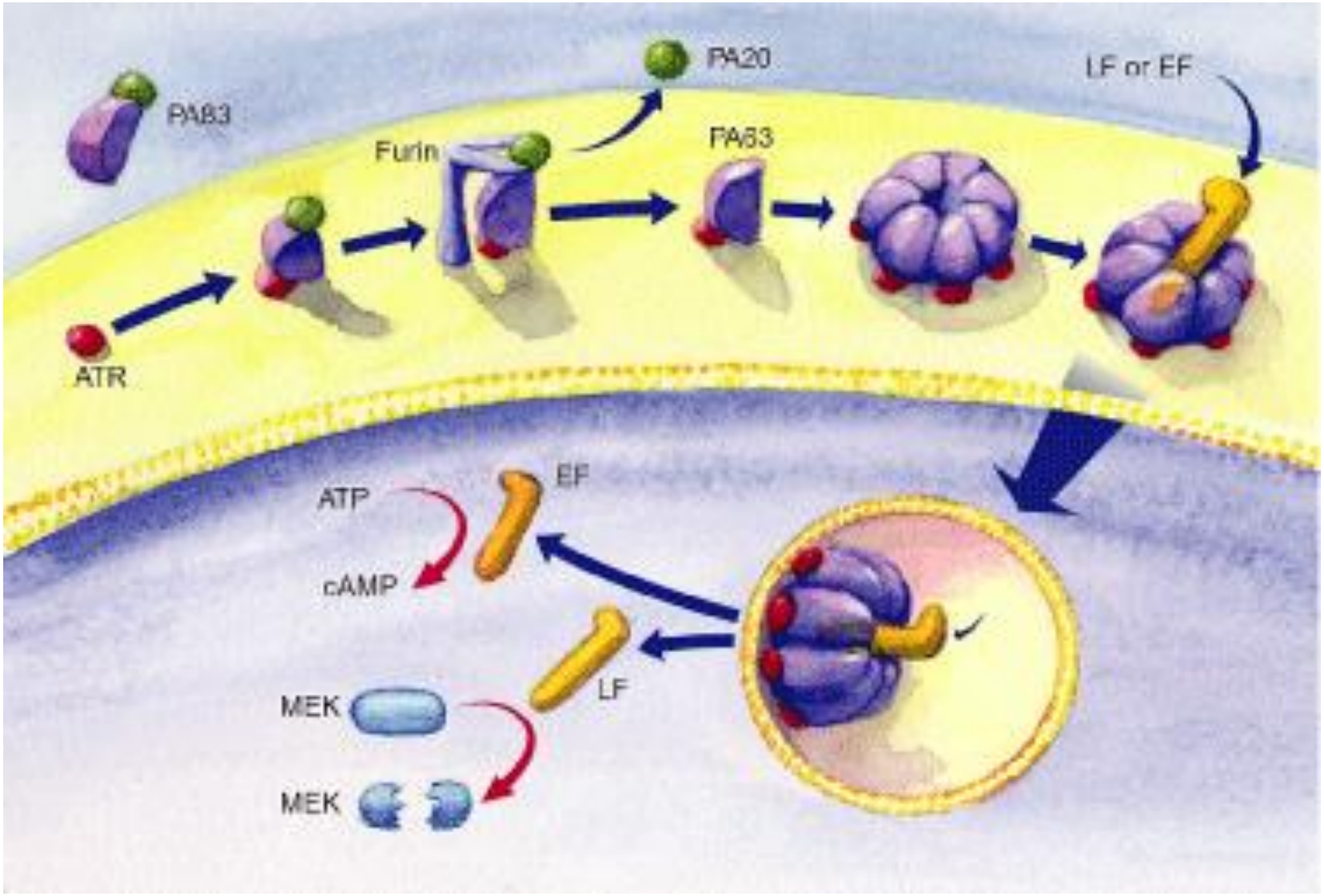
Antigenic properties

- Contains a thermostable polysaccharide somatic antigen composed of cell wall D-galactose and N-acetylglucosamine. Antibodies are not protective against this antigen. It persists for a long time in cultures and cadaveric material. The Ascoli thermoprecipitation reaction is based on its detection.
- ***Capsule antigen*** protein origin, represented by a polypeptide, which includes D-glutamic acid. Antibodies to capsular antigens are not protective.

Pathogenicity factors:

- ***Protein toxin (anthrax toxin)***protective antigen, lethal and edematous factors. Individually, these components are not capable of exhibiting a toxic effect:
- ***Protective antigen has no toxic effect,*** it forms pores in the membranes of human cells, which allows edematous and lethal factors to enter the cell.
- ***lethal factor*** exhibits a cytotoxic effect
- ***Edema factor*** exhibits the effect of adenylate cyclase, increases the concentration of intracellular cAMP and causes the development of edema of various tissues.

Mechanism of action of B.anthracis toxin



Source of infection and routes of transmission

- The source of infection are sick animals, in particular cattle, as well as sheep, goats, horses, deer, buffaloes, camels and pigs.
- ***The main clinical signs of the disease in animals***– convulsions and diarrhea with an admixture of blood.
- A person becomes infected most often by contact, less often by alimentary, aerogenic and other ways, when caring for sick animals, slaughtering, processing animal raw materials, eating meat and other livestock products.
- A sick person is not a source of infection.

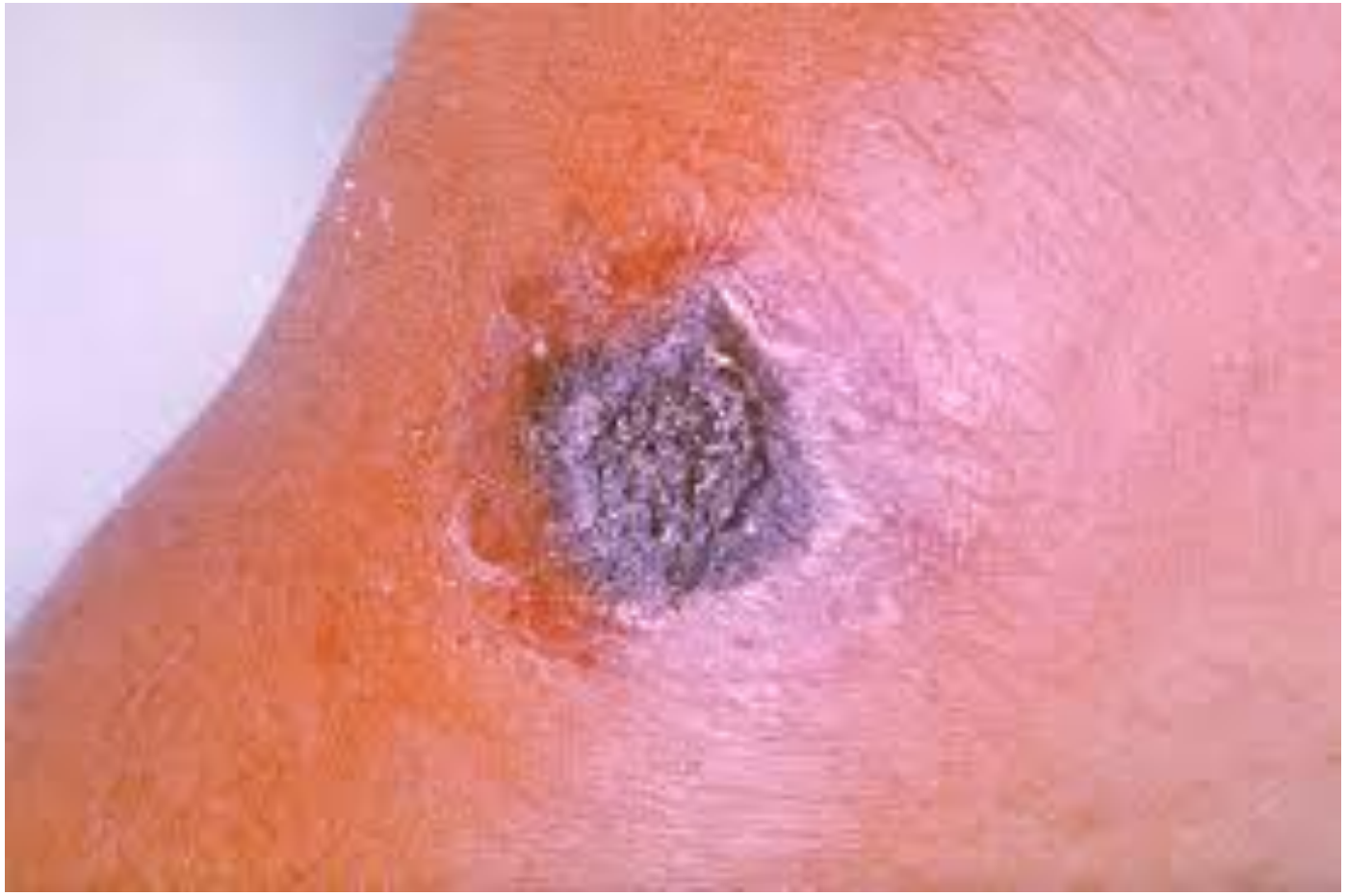
Pathogenesis and clinical manifestations of anthrax:

- *B.anthracis penetrates through damaged skin, mucous membranes of the respiratory tract and gastrointestinal tract. The duration of the incubation period is 2-6 days.*
- The pathogen from the entrance gate is introduced by macrophages into the regional lymph nodes, in which inflammation develops without serious violations of the barrier function, due to which the generalization of the process does not occur or occurs at a relatively late date.
- Dissemination in all clinical forms of the disease is accompanied by bacteremia and meningitis..
- Generalized forms in 100% of cases end in death.

Clinical forms of anthrax:

- ***The cutaneous form*** *is common (approximately 95% of cases). Anthrax carbuncle develops at the site of pathogen penetration into the skin - a focus of hemorrhagic-necrotic inflammation of the deep layers of the dermis at the border with subcutaneous tissue, accompanied by edema and tissue destruction.Легочная форма* (5% случаев)
- ***Gastrointestinal form (very rare)***
- **Septic form -** dissemination is possible, accompanied by bacteremia and meningitis.

Anthrax carbuncle



Microbiological diagnosis of anthrax:

- Compliance with all safety measures when working with especially dangerous infections.
- Materials of the study - the contents of the carbuncle, sputum, feces, blood and urine. According to epidemiological indications, various objects of the external environment, as well as animals, are examined.

Microbiological diagnosis of anthrax:

- ***Microscopic method****. Gram-stained smears prepared from the contents of the carbuncle and the blood of dead animals reveal large gram-positive bacilli arranged in chains.*
- ***Fluorescent microscopy*** *is used to detect anthrax bacilli in a smear treated with luminescent serum.*
- ***Bacteriological method.*** *The test material is sown on simple nutrient media to isolate a pure culture with its subsequent identification.*

Microbiological diagnosis of anthrax:

- ***Biological sample*** *- samples with the test material or isolated pure culture are placed on white mice and guinea pigs in order to detect the pathogen.*
- ***Serological method*** *- to detect specific antibodies in the patient's blood serum, the reaction of latex agglutination and RPHA is used. Recently, RIF has been used to determine antibodies to a lethal toxin and a factor that causes edema.*
- ***Skin allergy tests with anthraxin.***

Microbiological diagnosis of anthrax:

- **The Ascoli thermoprecipitation reaction** is set for the presence in the test material (skins, organs of a dead animal, decomposed or mummified animal corpse, skin and products from it) of an anthrax thermostable polysaccharide antigen.

Prevention includes non-specific preventive measures

- ***Non-specific preventive measures*** *are reduced to sanitary and veterinary measures.*
- *Isolation of sick and suspicious animals, sanitary supervision of enterprises engaged in the processing of animal raw materials, disinfection of places where sick animals are kept, and other measures.*

Specific prevention:

- In the USA and England, a cell-free vaccine is produced from avirulent strains of the pathogen adsorbed on aluminum hydroxide. The vaccine contains all components of the anthrax toxin.
- Immunization is carried out according to epidemic indications to persons of a high-risk group 3 times every two weeks. Then revaccination is carried out after 6,12 and 18 months. Then immunization is carried out once a year.

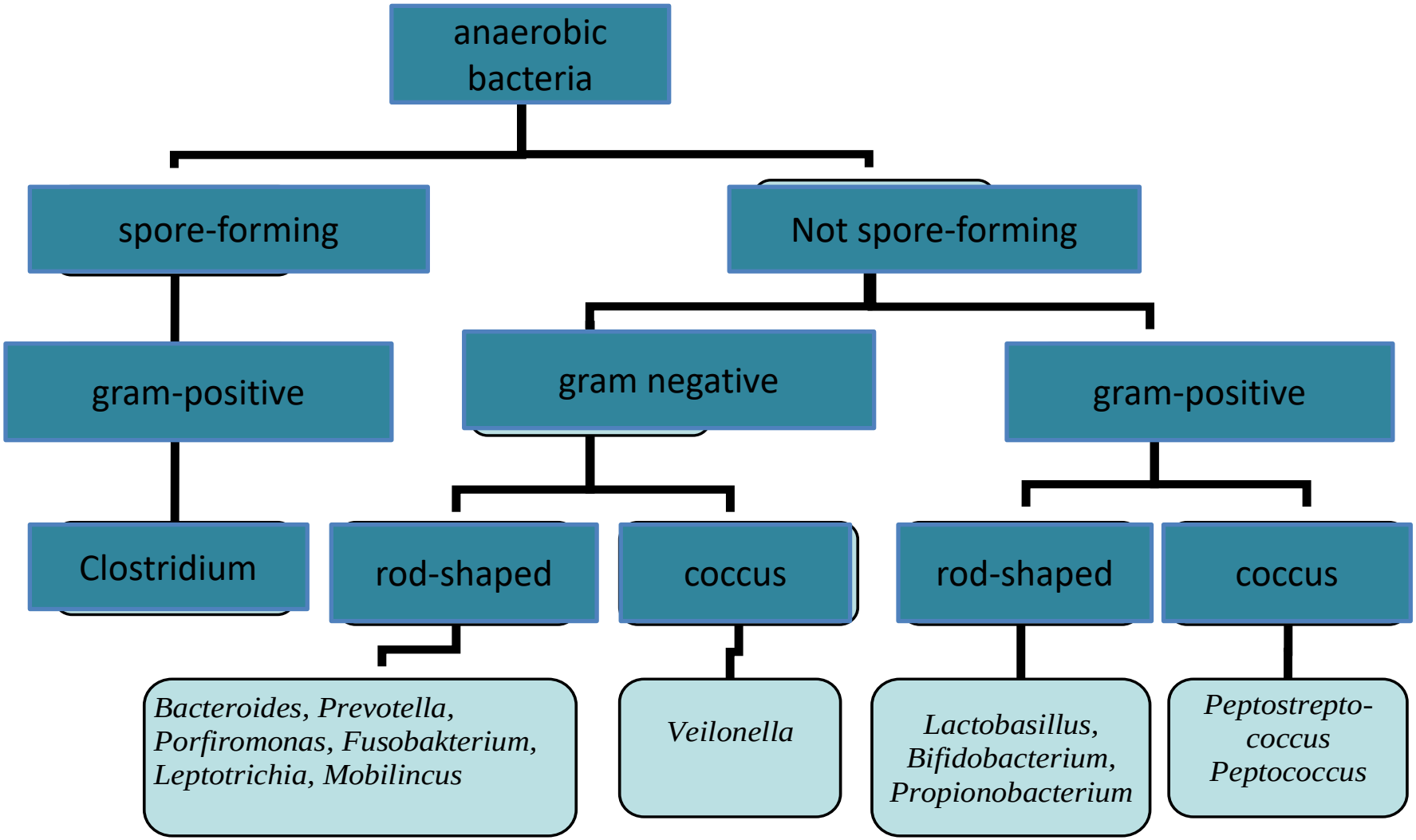
Chemical prophylaxis:

- Along with vaccination, chemical prophylaxis is carried out to prevent mass infection and the use of ciprofloxacin or doxycycline for 4 weeks.
- Chemical prophylaxis is carried out for unvaccinated persons for 8 weeks.

Treatment

- Recently, ciprofloxacin has been prescribed in the treatment of anthrax.
- Combinations of penicillin G with gentamicin and streptomycin are also used.

Classification of anaerobic bacteria

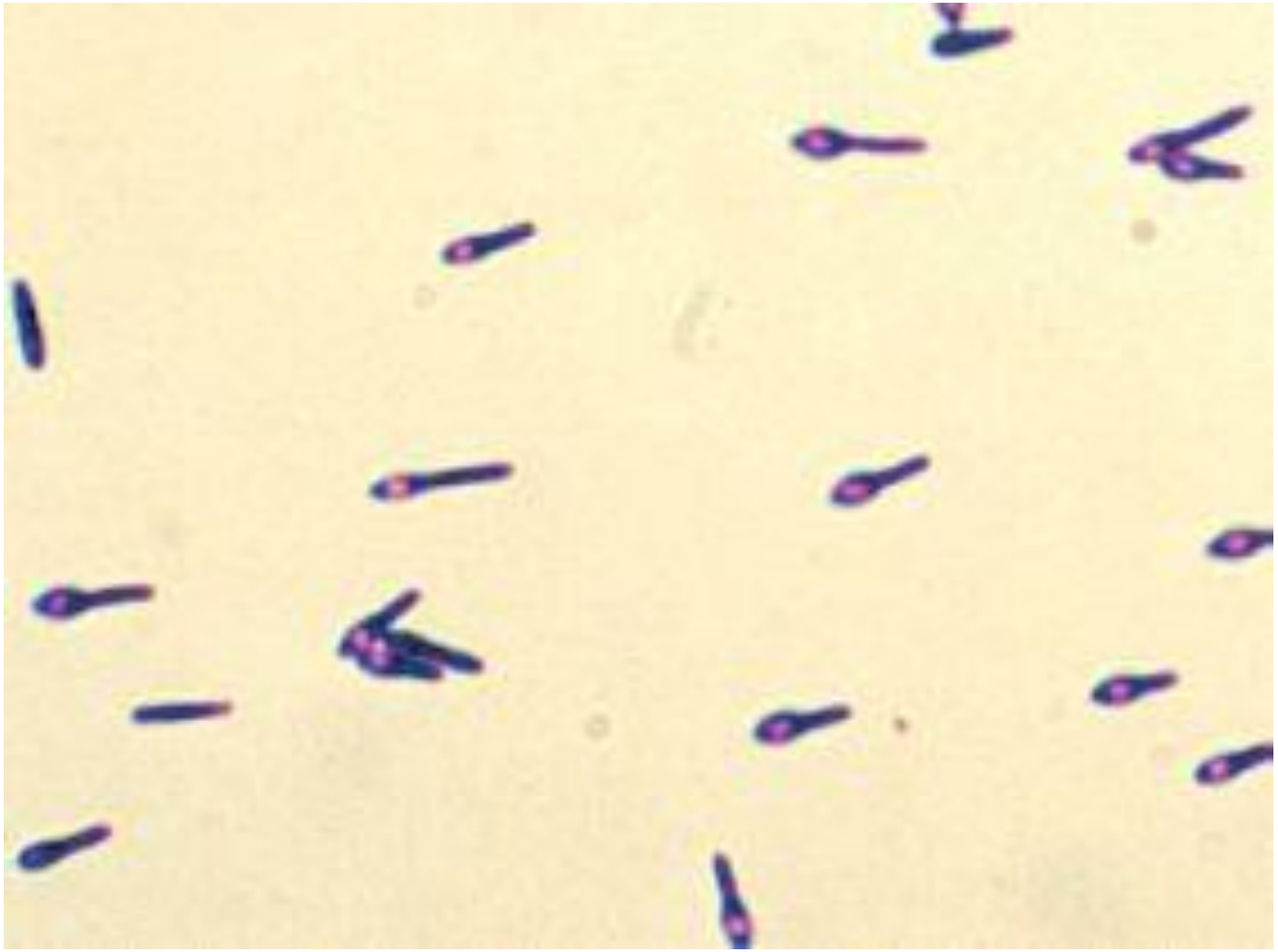


Clostridium botulinum

Morpho-biological properties:

C.botulinum – movable polymorphic rods with rounded ends, 4-9x0.6-1.0 μm in size. Young cultures stain gram positive, old cultures stain gram negative. The capsule does not form. Under unfavorable conditions, they form spores located subterminally, which gives the bacteria the shape of a "tennis racket"

Clostridium botulinum



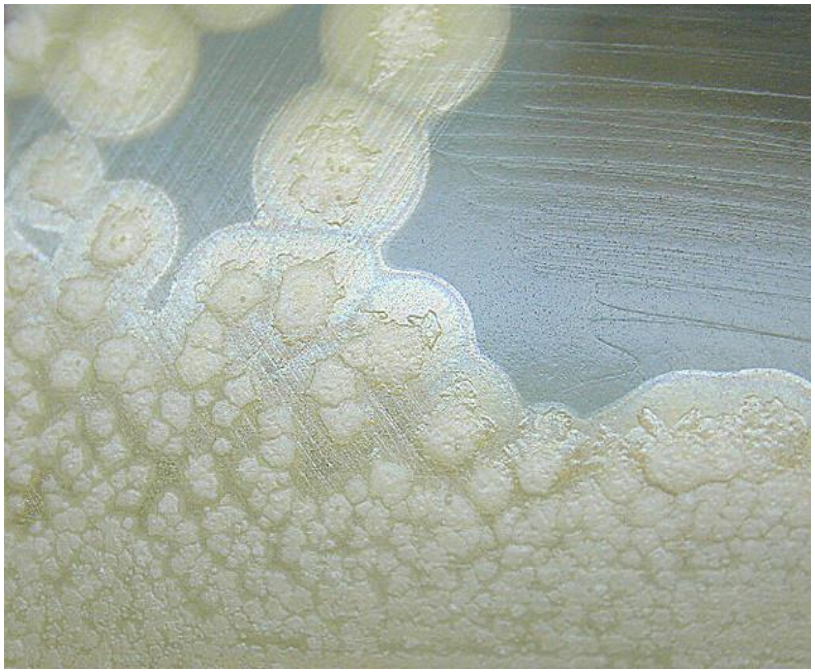
Morpho-biological properties

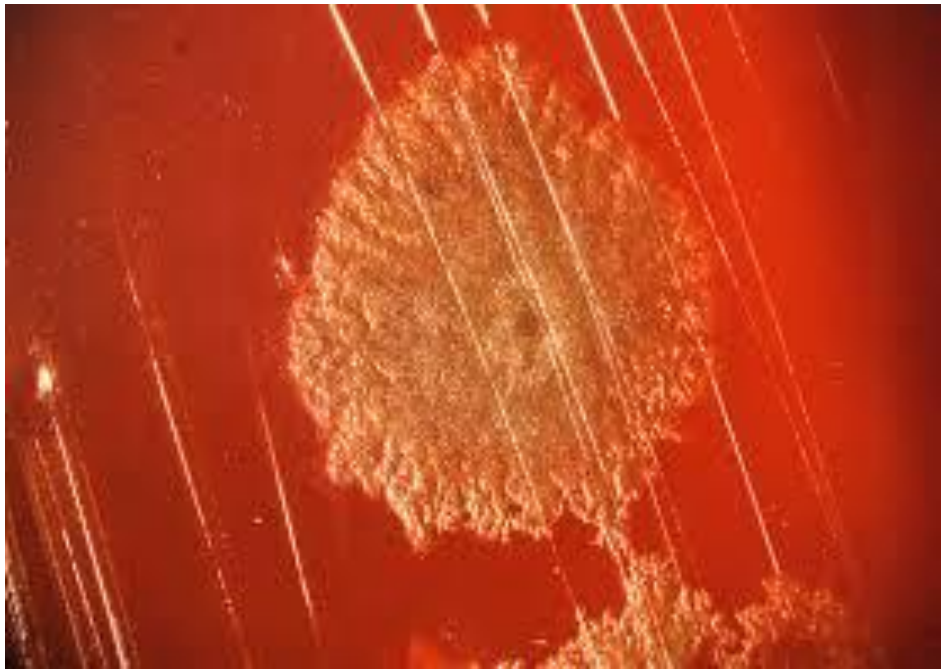
Obligate anaerobes. On blood agar with glucose, they form irregularly shaped colonies with filamentous branches and a zone of hemolysis. On blood agar, they form S-colonies in the form of dew drops, or R-colonies with jagged edges. On liquid media (Kitt-Tarozzi medium, liver broth) cause clouding of the medium and gas formation.

Biochemically active: ferment carbohydrates to acid and gas, ferment milk, form gelatinase, lecithinase, hydrogen sulfide and ammonia.

Clostridium botulinum

colonies





Antigenic structure

Serological identification of C.botulinum is based on the identification of the exotoxin produced by them; according to their antigenic structure, bacteria are divided into 8 serovars: A, B, C1, C2, D, E, F and G.

Ecology, source of infection and transmission routes:

- *C.botulinium is widely distributed in soil and water (contamination from animal and fish feces).*
- *From the soil, spores can get to food (in particular, meat, fish, home-made canned vegetables), where, under anaerobic conditions, they germinate and release exotoxin. Eating these foods leads to severe food poisoning - botulism.*
- *Types A, B, and E are the most common types. Type F rarely causes disease in humans. Type E bacteria mainly cause disease when fish products are eaten.*

Eating canned meat, fish and vegetable products pose the greatest risk of botulism!

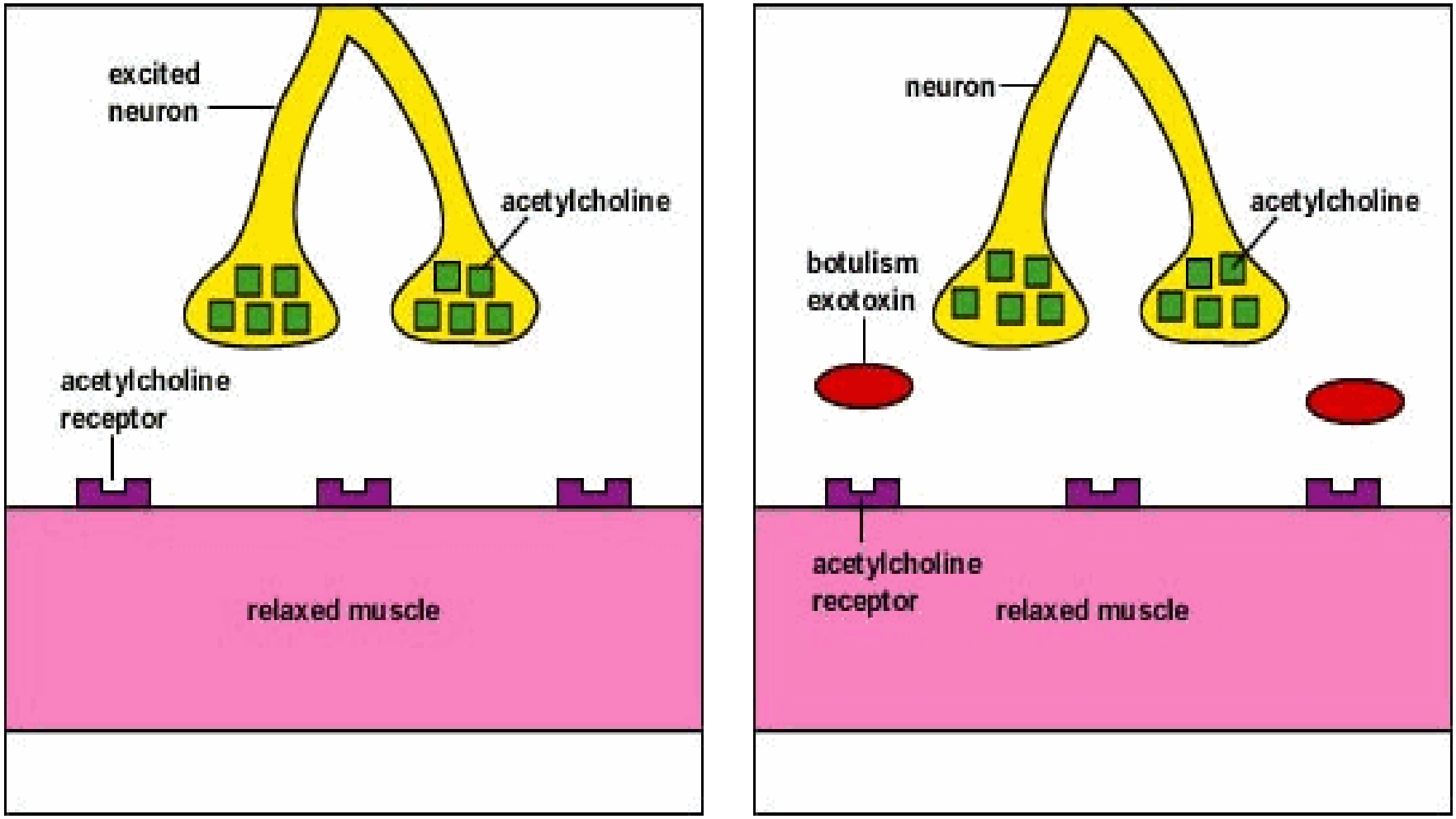
Pathogenic factors:

- ***C.botulinum in the process of reproduction or during autolysis produce an exotoxin - botulinum toxin.*** Botulinum toxin - a protein with a molecular weight of 150 kDa consists of two protein subunits linked by a disulfide bond (heavy and light chains). The toxin is thermolabile, boiling for 20 minutes is necessary for complete inactivation.

The action of botulinum toxin

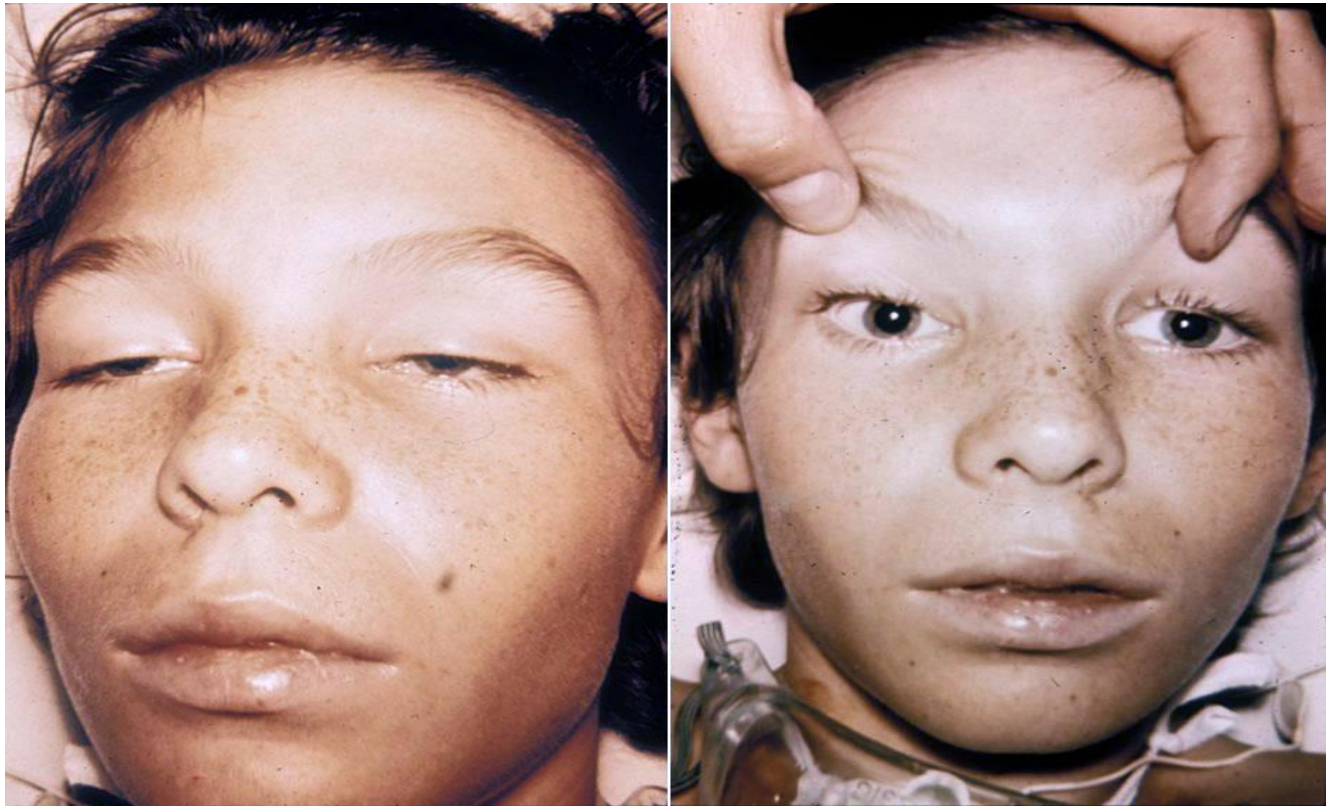
- Botulinum toxin is a functional blocker. The absorption of botulinum toxin occurs in the stomach and partly in the small intestine, from where the toxin enters the organs and tissues with the bloodstream. Botulinum toxin binds to receptors on the surface of the presynaptic membrane of motor neurons in the peripheral nervous system and enters the neurons by endocytosis.
- The light chain (A-component) of botulinum toxin, which is responsible for toxigenicity, blocks the release of acetylcholine in the neuromuscular endings of the motor nerves (it breaks down synaptobrevin, syntaxin, cellubrevin), which causes characteristic muscle paralysis.

Mechanism of action of botulinum toxin



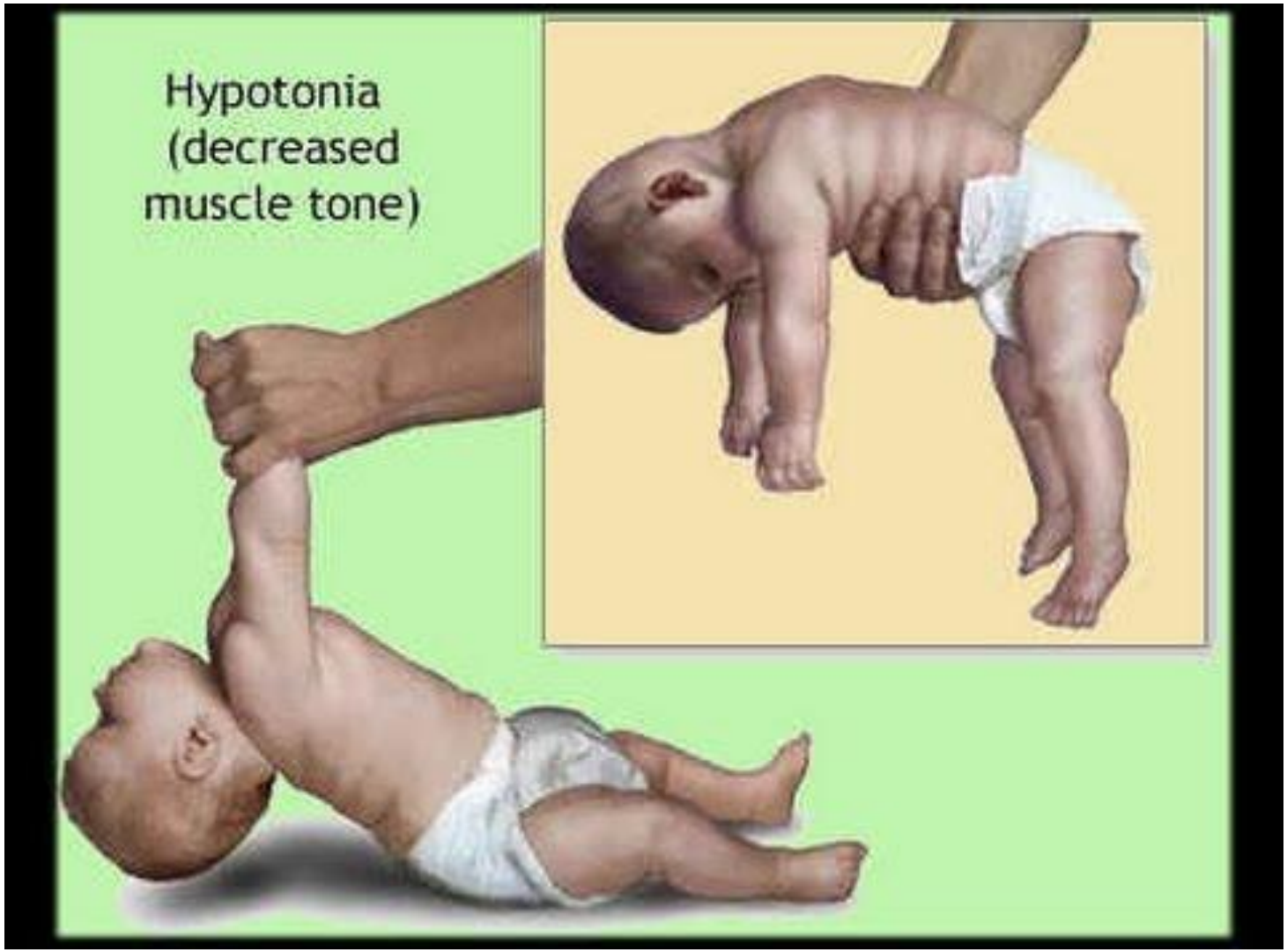
Clinical manifestations of botulism

nerve phenomena - impaired swallowing, diplopia (double vision), ptosis (drooping of the eyelids), anisocoria (damage to the sphincter of the pupil).



Eyelid drooping - ptosis

Botulism in infants



Microbiological diagnostics:

- Food residues, vomit, gastric lavage, feces, urine, blood, sectional material are subject to research.
- Biological test - carried out to detect a toxin on white mice or guinea pigs
- Neutralization reaction - determination of botulinum toxin and its type: white
- mice are injected with a mixture of the test material with diagnostic
- antitoxic serum types A, B, E and F.
- Passive hemagglutination reaction, radioimmune method - allows you to determine botulinum toxin in the test material.
- Isolation of the culture of the pathogen - is carried out to clarify the diagnosis of botulism in infants using the bacteriological method for examining feces.

Treatment:

- ***For treatment, the patient is intravenously injected (according to Bezredko) polyvalent (types A, B and E) antitoxic serum.***
- *After laboratory identification of the type of pathogen, serum is administered only against this type.*

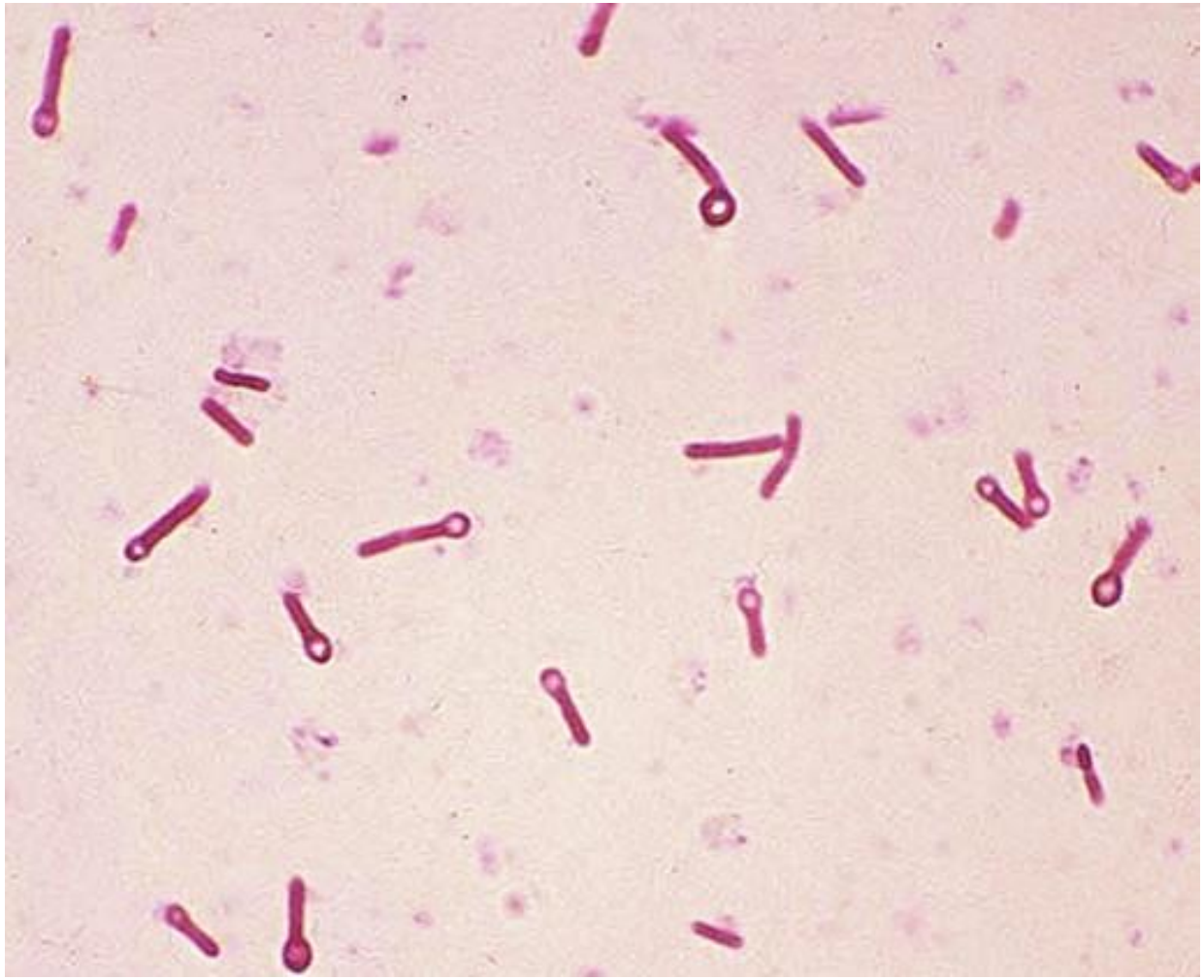
Prevention

- **Non-specific prophylaxis -** in the production of canned (meat, fish, vegetable) products, it is necessary to observe sanitary and hygienic conditions for sterilization and storage, which exclude the accumulation of toxin in the product.
- **Specific prophylaxis -** use botulinum polyanatoxin containing toxoids A, B and E.
- For emergency prophylaxis, polyvalent antitoxic serum is used.

Morpho-biological properties of Clostridium tetani

C.tetani **–** Gram-positive rods with rounded ends, 4-8 μm long and 0.4-1.0 μm thick. Possess mobility due to peritrichous flagella; form spores of a round, less often oval shape, located terminally and giving the bacteria the appearance of a "drum stick". Do not form a capsule.

Clostridium tetani

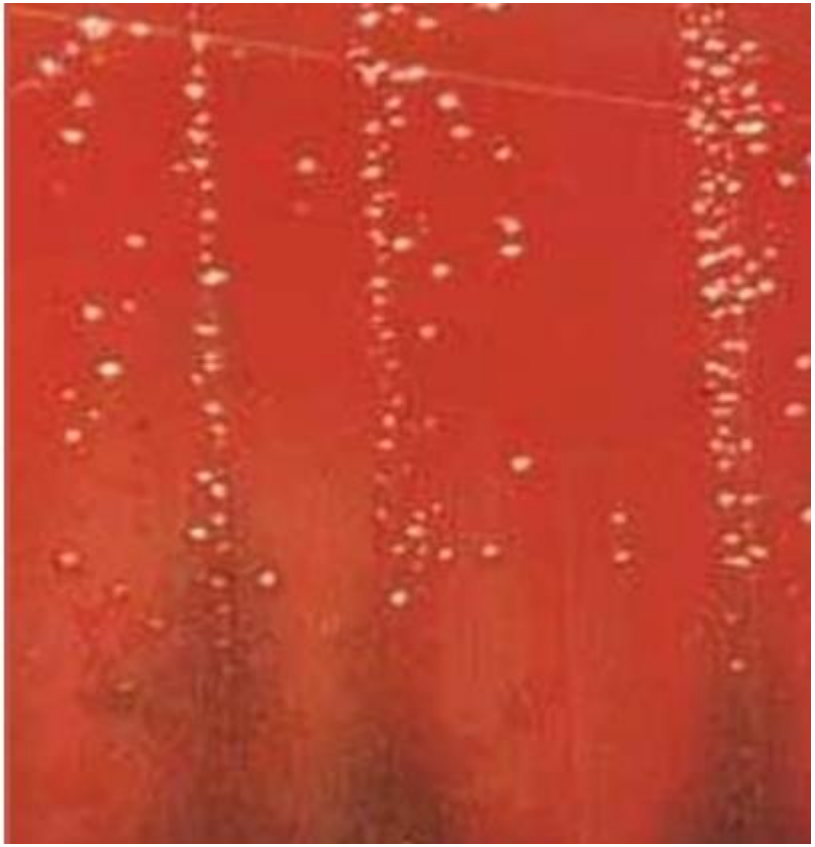


Morpho - biological properties

Obligate anaerobes, grow well on media under anaerobic conditions. On Kitt-Tarozzi media, they form turbidity; on Wilson-Blair media, they cause blackening of the medium.

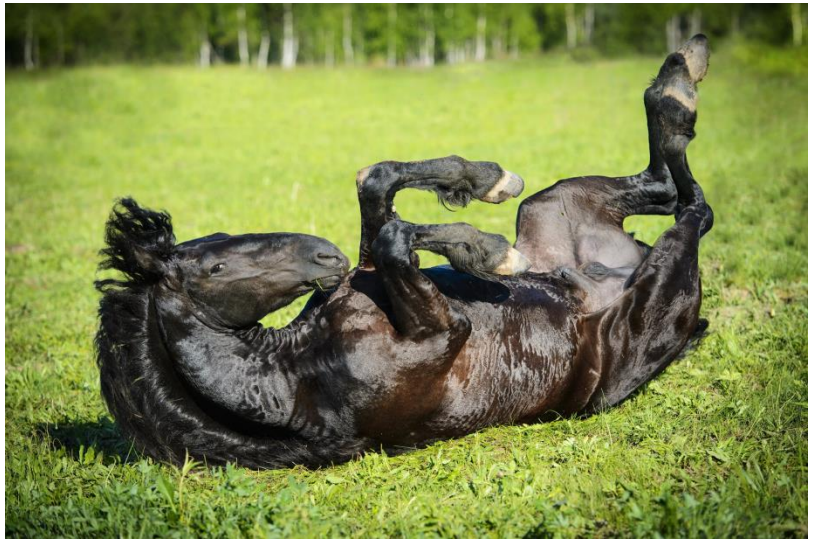
Possess weak biochemical activity. Do not ferment carbohydrates, exhibit weak proteolytic properties. Slowly curdle the milk and liquefy the gelatin.

Clostridium tetani *colonies on blood agar*



Animal susceptibility to tetanus

- Under natural conditions, horses and small cattle suffer from tetanus.
- Of the experimental animals, white mice, rats and rabbits are susceptible to the causative agent of tetanus.
- Tetanus in animals occurs with the phenomena of spastic contractions of the striated muscles and damage to the pyramidal cells of the anterior horns of the spinal cord. First, the limbs are involved in the process, and then the trunk (ascending tetanus). Death occurs due to paralysis of the heart muscle.



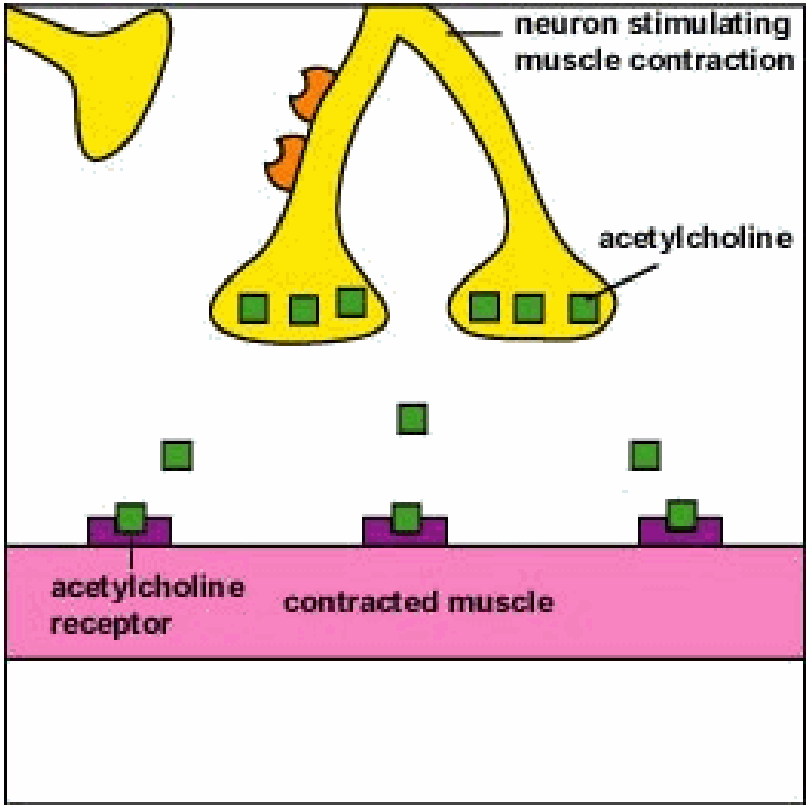
Source of infection and routes of transmission

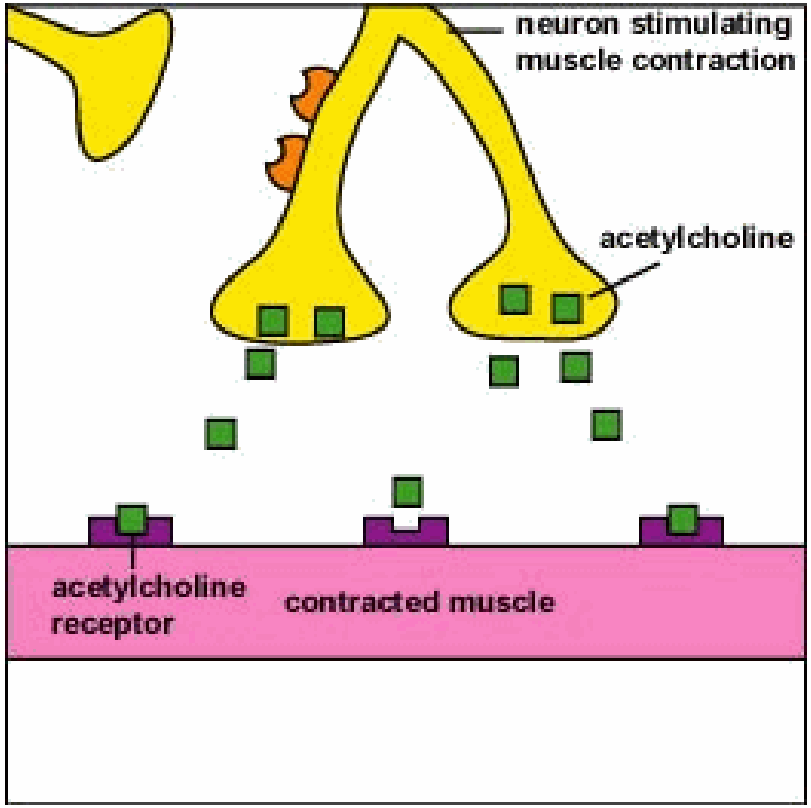
- Entrance gates of infection - damaged skin and mucous membranes
- Tetanus is an acute wound toxinemia infection. More than half of the cases are people living in rural areas, agricultural workers involved in the construction of roads, houses, etc.
- The greatest danger is represented by deep, blind and axillary wounds contaminated with soil, where favorable conditions are created for the development of anaerobic infection.

Pathogenicity factors and pathogenesis

- ***Tetanospasmin (exotoxin)*** enters the blood from the primary site of infection. By binding to receptors on the presynaptic membrane of motor neurons, it penetrates into them and, through retrograde axon transport, enters the central nervous system, where it can penetrate into inhibitory neurons. The mechanism of action of the toxin is associated with the suppression of the release of inhibitory neurotransmitters, in particular glycine and γ-aminobutyric acid, which causes overexcitation of motor neurons and leads to spastic paralysis, hyperreflexia and convulsions.

The mechanism of action of tetanospasmin





Clinical manifestations of tetanus:

Immunity

- Since the toxigenic dose of tetanus toxin is many times lower than the immunogenic dose, immunity is not formed and repeated cases of the disease are noted.

Microbiological diagnostics:

With tetanus, usually microbiological diagnosis is not carried out in view of the pronounced picture of the disease.

- On the other hand, it is not always possible to detect the pathogen at the site of the entrance gate of infection.
- Of great importance in identifying the entrance gate of infection are anamnestic data.

Treatment

- Specific treatment is aimed at neutralizing the tetanus toxin with an antitoxin.
- For this purpose, anti-tetanus antitoxic serum or anti-tetanus immunoglobulin is used.

Specific prophylaxis

- To create artificial active immunity, tetanus toxoid (toxoid) sorbed on aluminum hydroxide as part of DTP, ATP or sextanatoxin vaccines is used. .

Specific prophylaxis

- Emergency prophylaxis is carried out for injuries, burns and frostbite, animal bites, and community-acquired abortions by introducing tetanus toxoid. Anti-tetanus serum is administered to those who are not vaccinated along with tetanus toxoid.

Causative agents of gas gangrene

- Anaerobic infections are caused by bacteria of the genus Clostridium of the Bacillaceae family. Usually the disease is caused by the action of several types of pathogens of the genus Clostridium in association with various aerobic bacteria (staphylococci, streptococci, etc.).
- The main causative agent of gas gangrene (approximately 90%) is ***C.perfringens.***

Other causative agents of gas gangrene:

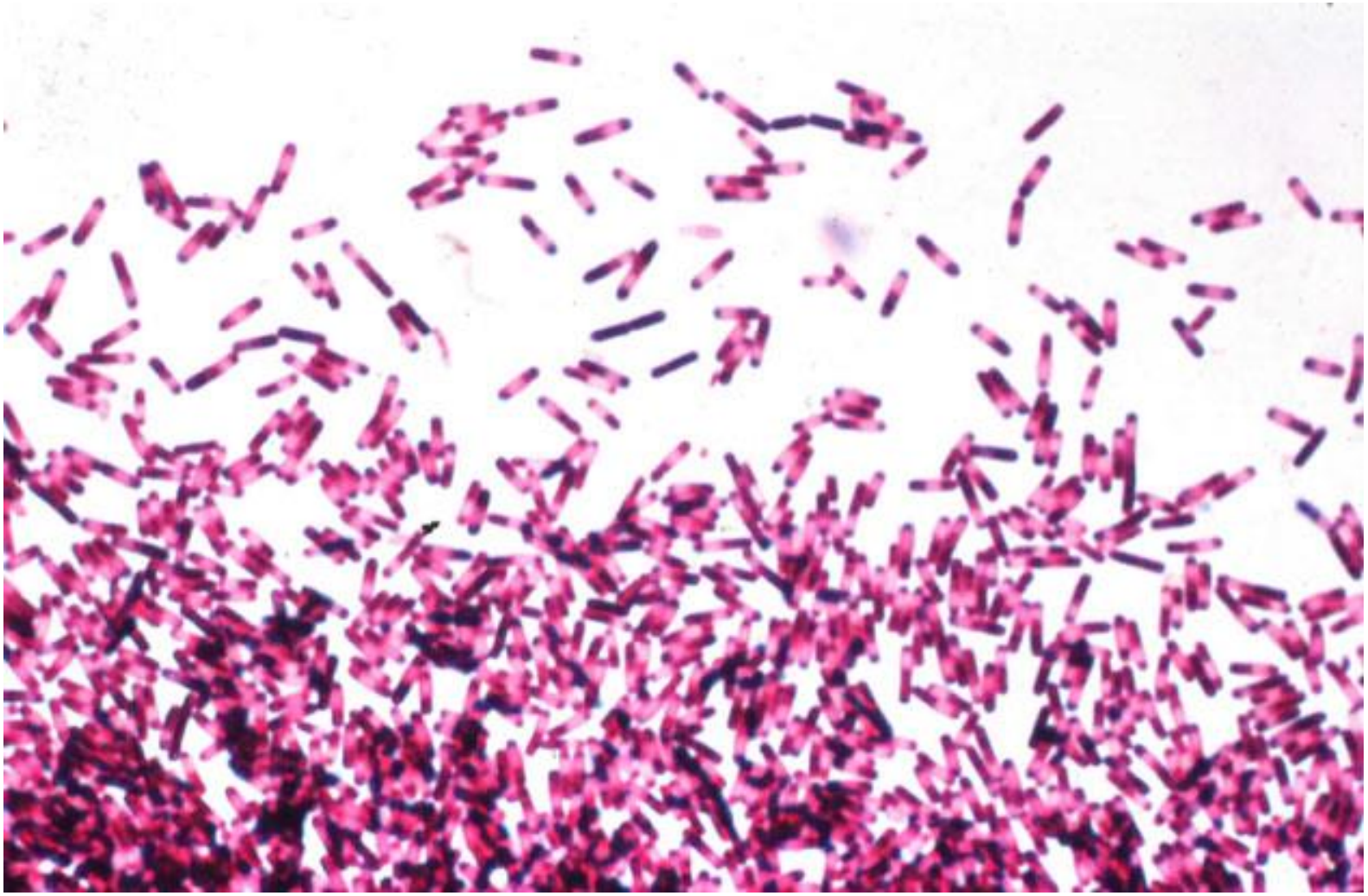
- ***Clostridium novyi, Clostridium septicum, Clostridium histolyticum*** **-** mobile, due to peritrichous flagella, which distinguishes them from ***C. perfringens;*** do not form a capsule**.**
- ***Clostridium sordellii***

Morpho-biological properties of Clostridium perfringens

***C. perfringens* –** large, gram-positive, immobile, polymorphic rods that form capsules in the body. They form spores located centrally or subterminally.

Biochemically active. Break down carbohydrates to form acid and gas. Proteolytic activity is weak. The milk is intensively curdled with the formation of a coarse spongy clot resembling foam on the waves. This phenomenon is known as ***storm reaction***.

Clostridium perfringens



Cultural properties:

- ***C.perfringens*** are aerotolerant anaerobes.
- They grow on nutrient media prepared from meat hydrolyzate and casein at a temperature of 37-420C, pH 7.2-7.4 for 3-8 hours.
- Growth on liquid nutrient media is manifested by ***turbidity*** and intense ***gas formation.***
- When growing in the depth of the agar tube, it causes a rupture of the column of the medium due to the rapid formation of gas.
- On dense nutrient media with the addition of blood, it forms colonies with a double zone of hemolysis. Around the colonies, complete hemolysis is formed due to the action of hemolysins, at a distance - incomplete due to the action of lecithinase.

Enzymatic activity:

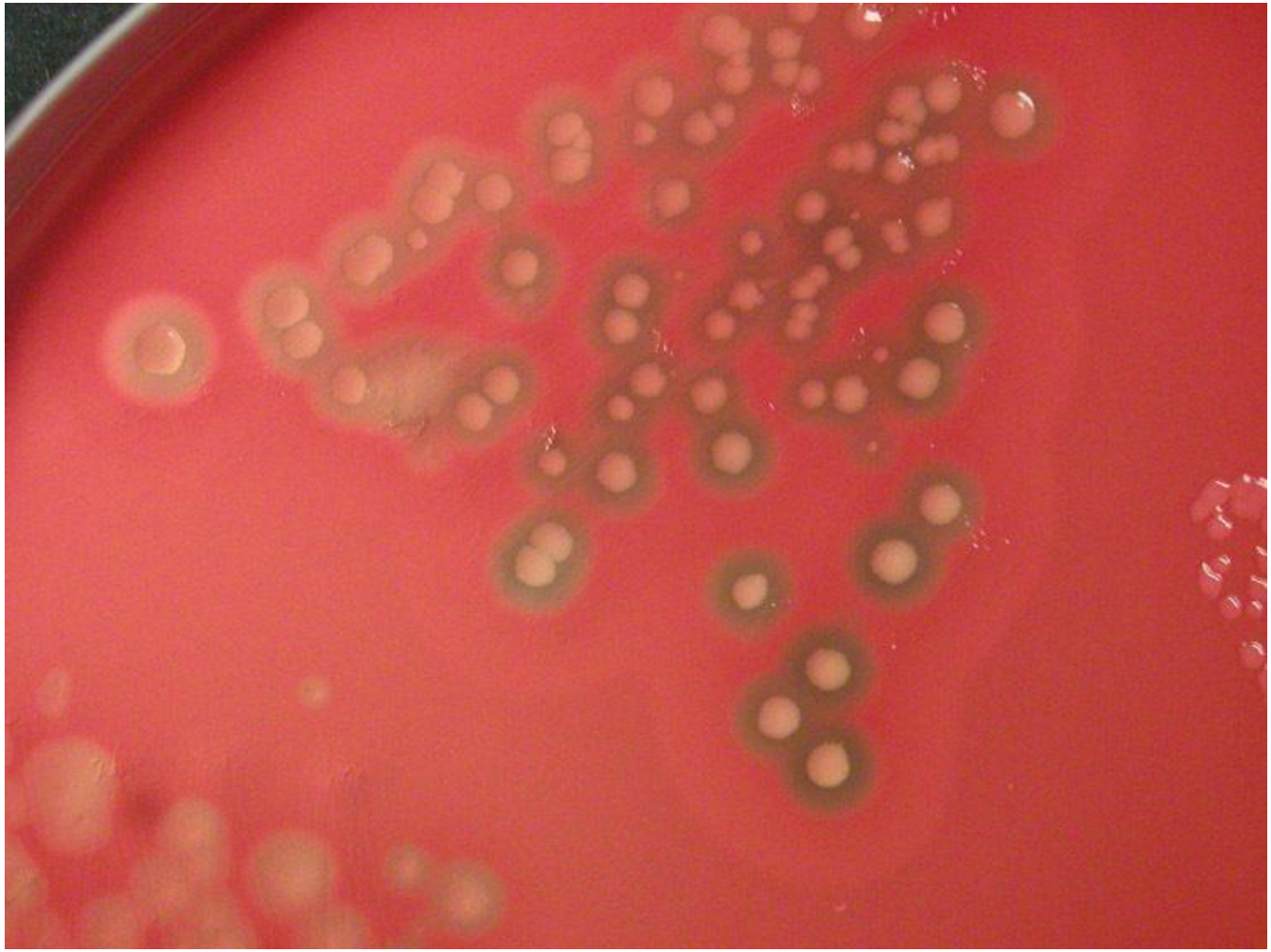
- C.perfringens has a high biochemical, especially saccharolytic activity.
- Carbohydrates - lactose, glucose, sucrose, maltose, xylose, galactose, mannose, starch and glycogen splits with the formation of acid and gas, does not ferment mannitol and dulcit.
- It differs from other clostridia in its ability to reduce nitrates, break down lactose and form lecithinase.
- Proteolytic activity is weak - it intensively curdles milk with the formation of a large-celled spongy clot ("**storm reaction**"), slowly liquefies gelatin, does not decompose casein.

Clostridium perfringens

(growth on Kitt-Tarozzi medium)

Clostridium perfringens

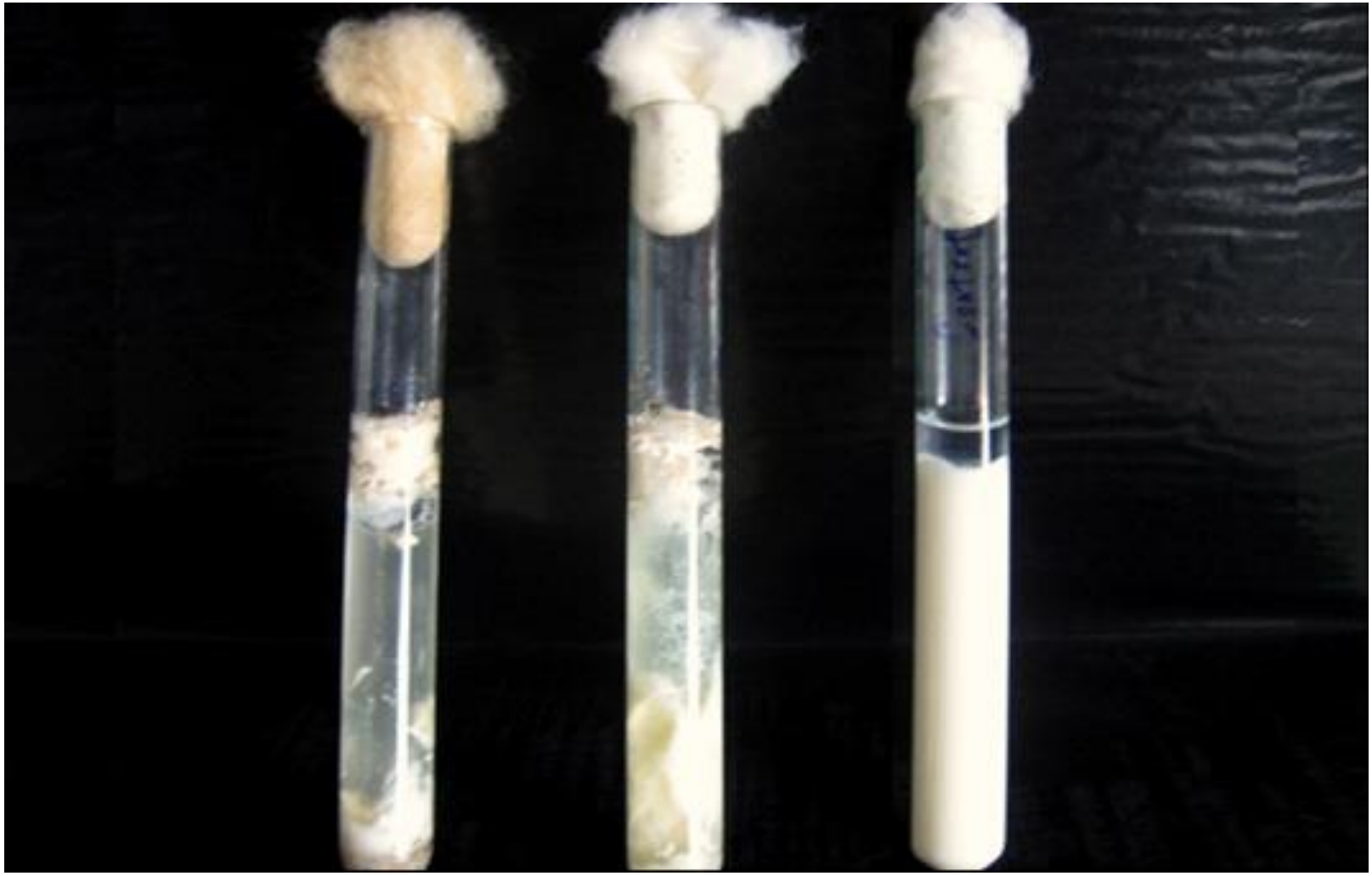
(colonies on blood agar)



Clostridium perfringens

(rupture of the agar column due to the formation of gas)

C.perfringens curdles milk into a large-mesh spongy clot within 3 hours ("storm reaction")



Antigenic structure:

- There are 6 serotypes - A, B, C, D, E, F, differing in the antigenic properties of the produced exotoxins.
- Serotype **A is an inhabitant of the normal intestinal microflora, but can cause gas gangrene and food poisoning in humans.**
- Serotype **B causes intestinal disorders in lambs**
- Serotype **C causes necrotizing enterocolitis in humans and cattle**
- Serotype **D causes enterotoxinemia in animals**.

Pathogenic factors:

- The toxin has lethal, necrotic and hemolytic properties and combines at least 14 factors, denoted by Greek letters.
- ***Alpha (α)- toxin (phospholipase C) -*** produce all serotypes of C.perfringens, is the main factor in the pathogenicity of all pathogens of gas gangrene. **α**- toxin, being a cytotoxin, has lecithinase activity.
- ***Theta(θ)- toxin*** –perfringolysin, ***kappa(κ)- toxin*** – collagenase, ***mi(μ)- toxin*** – hyaluronidase, ***ni (ν)- toxin*** –DNA-ase, etc. individually, they do not have an effect, they only enhance the effect of ***α*** -toxin.
- *C. perfringens serotype* A form an enterotoxin that causes food poisoning.

Source of infections and transmission routes:

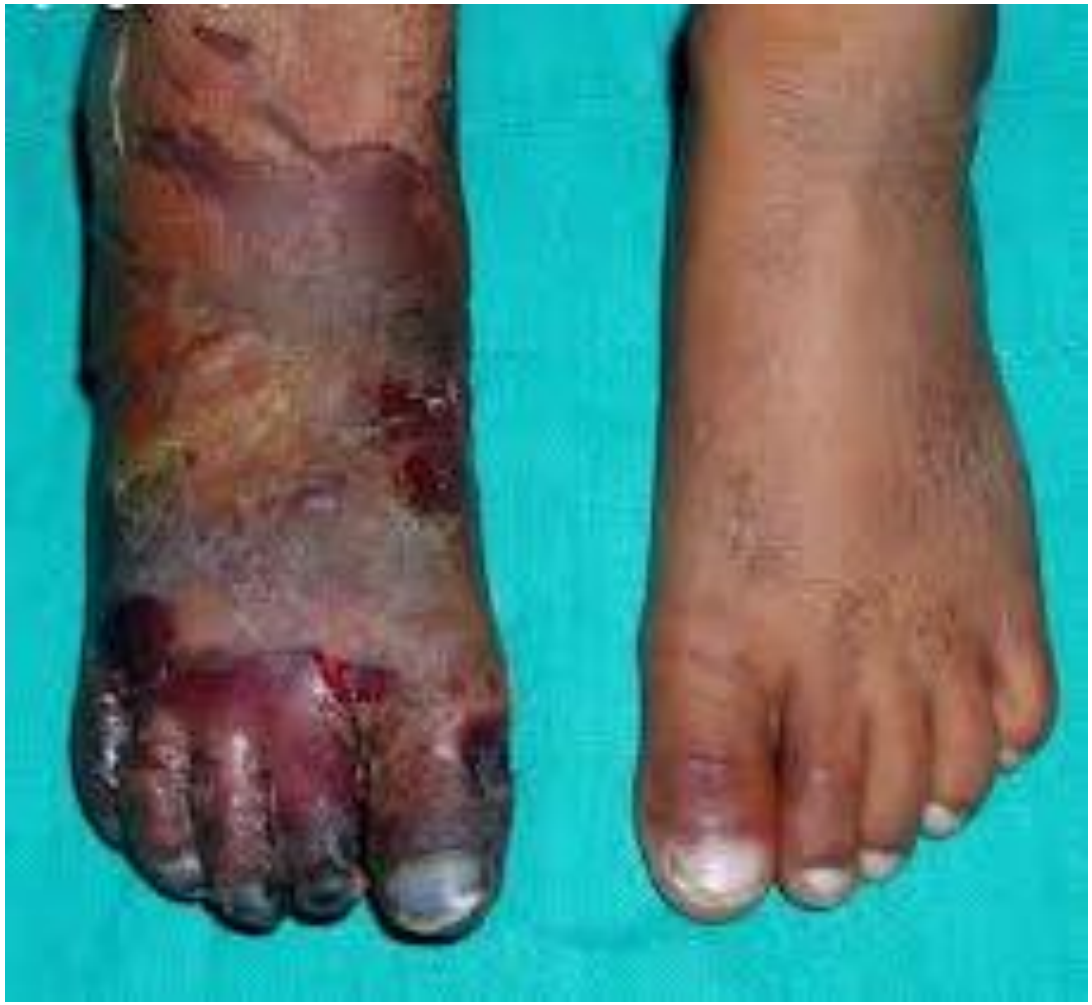
- The natural reservoir of the pathogen is the soil.
- Путь передачи - contact. Wound infection occurs when clostridia come in contact with soil or contaminated soil objects.
- Gas gangrene is most often observed in wounds where anaerobic conditions develop (in deep layers of muscle tissue, in wound pockets, necrotic tissues).
- Morbidity increases significantly during military operations among the wounded. In peacetime, builders, miners, and agricultural workers are more often infected. Infection with gas gangrene can develop even after car accidents, abdominal operations, out-of-hospital abortions

Clostridium perfringens

(diseases caused)

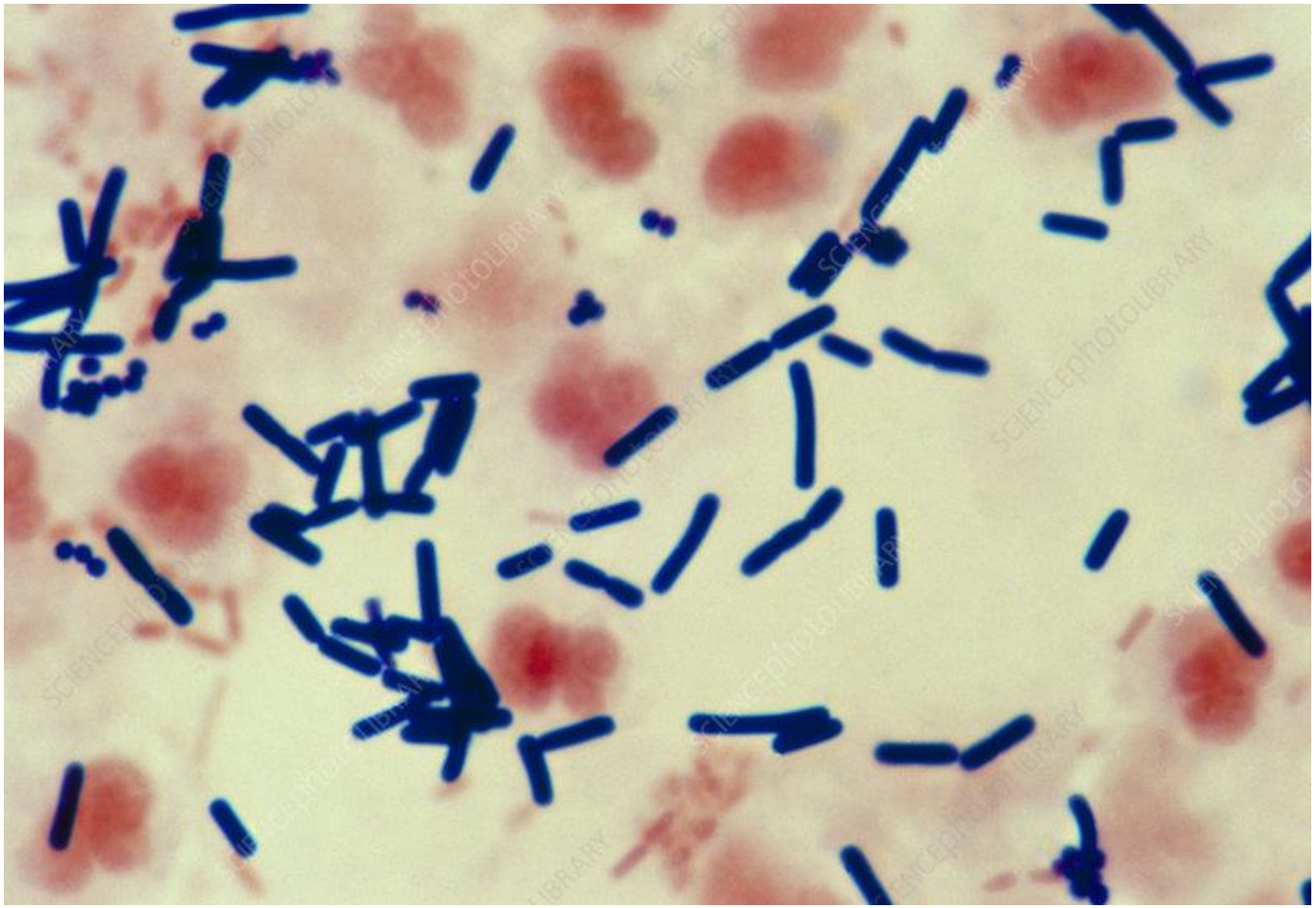
- ***Gas gangrene***
- ***Food poisoning (****develops when eating food contaminated with C.perfringens****)***
- ***Necrotic enteritis***

Clinical manifestations of gas gangrene



Clostridium perfringens

in a wound swab



Microbiological diagnosis of gas gangrene:

- **Materials for research**: pieces of affected tissue, exudate, pus, wound discharge.
- **Microscopic method**. The detection of large gram-positive rods stained by Gram in a smear is a diagnostic sign of the pathogen.
- **Bacteriological method**. Sowing the test material on thioglycol medium, Kitt-Tarozzi medium and blood agar to isolate a pure culture and identify the pathogen.

Microbiological diagnosis of gas gangrene

- To identify C. perfringens, inoculation into milk is carried out. Bacteria curdle milk with the formation of a coarse spongy clot within 3 hours.
- Lecithinase activity is determined in media containing egg yolk.
- Identification is completed by carrying out the reaction
- neutralization of toxins with specific
- antitoxic serums.

Naegler test - *at 35ºC under anaerobic conditions, lecithin-positive bacteria form opaque fields around the strokes.*

Prevention of gas gangrene

- **Nonspecific prophylaxis.** Surgical treatment of wounds, removal of necrotic tissues and foreign bodies, the use of antiseptics can largely prevent the development of the disease.
- **Specific prophylaxis.** Apply polyanatoxin containing toxoids of the most common pathogens of gas gangrene.
- For the purpose of emergency seroprophylaxis in case of injuries, a polyvalent antitoxic serum against gas gangrene is administered.

Treatment for gas gangrene:

- Antibiotics - penicillin G (benzylpenicillin)
- Specific treatment - ***antitoxic serum***

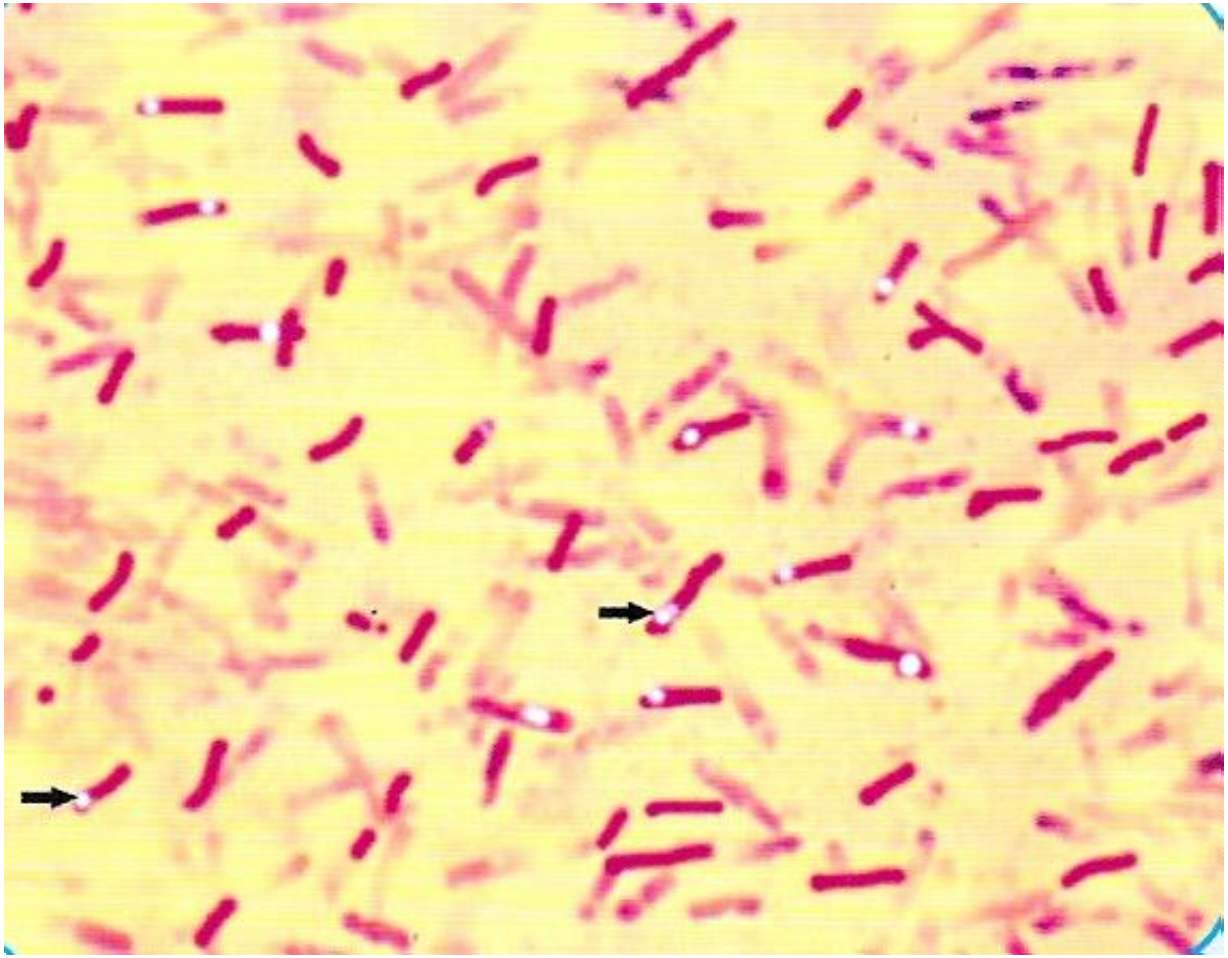
Morpho-biological features of Clostridium difficile

C.difficile - Gram-positive, oval, motile, rod-shaped bacteria. They form subterminally or centrally located spores. Form a capsule in vivo. obligate anaerobes. In a selective medium - on yolk-fructose agar with the addition of cycloserine and cefoxitin - they form cloudy, glassy yellow colonies. On blood agar, they form non-hemolytic colonies with a "horse manure" odor.

Biochemically little active. From carbohydrates, it breaks down glucose and mannitol, hydrolyzes gelatin, forms indole, does not have lecithinase and lipase activity.

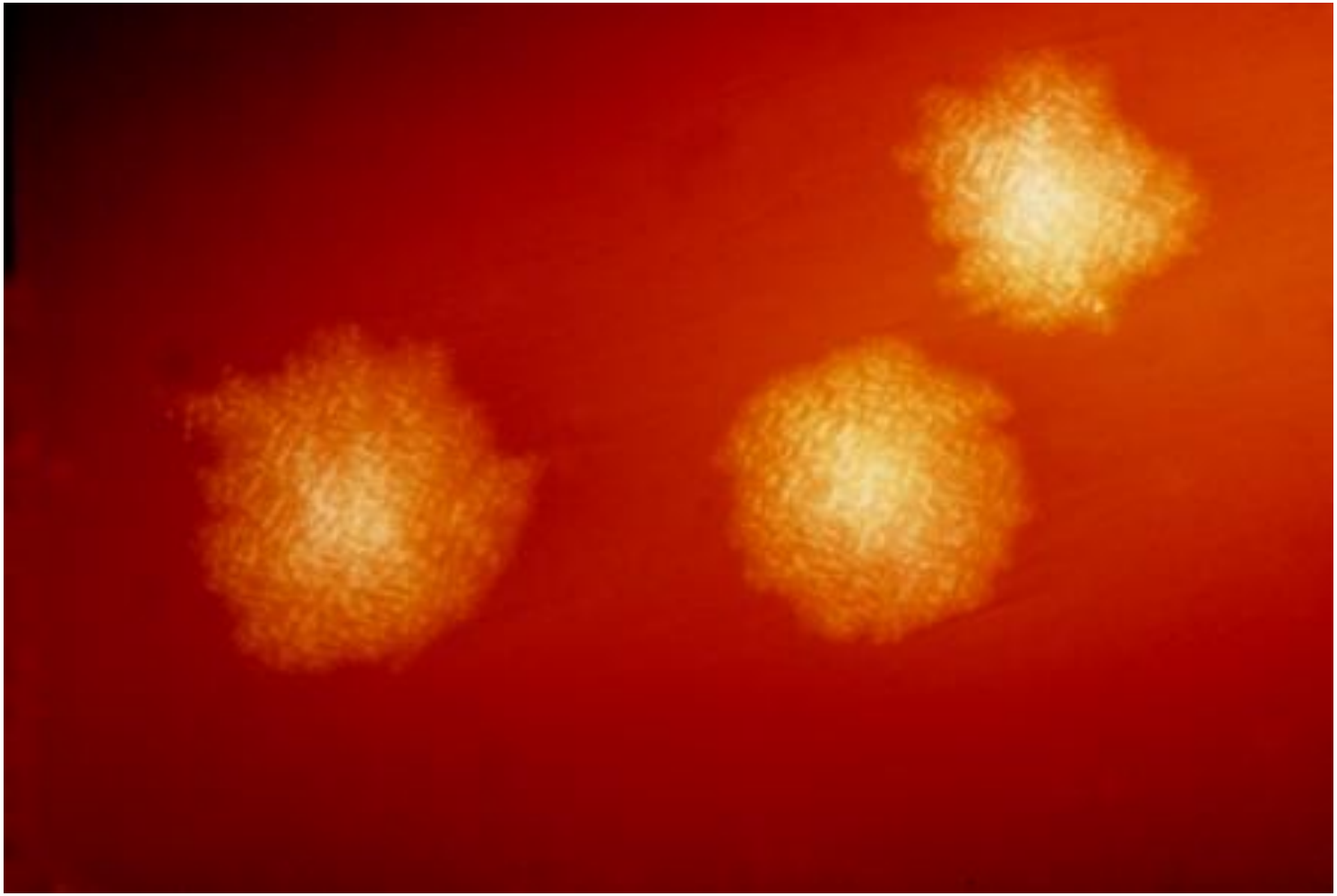
Clostridium difficile

(smear from pure culture)



Clostridium difficile

(colonies on selective medium)



Pathogenicity

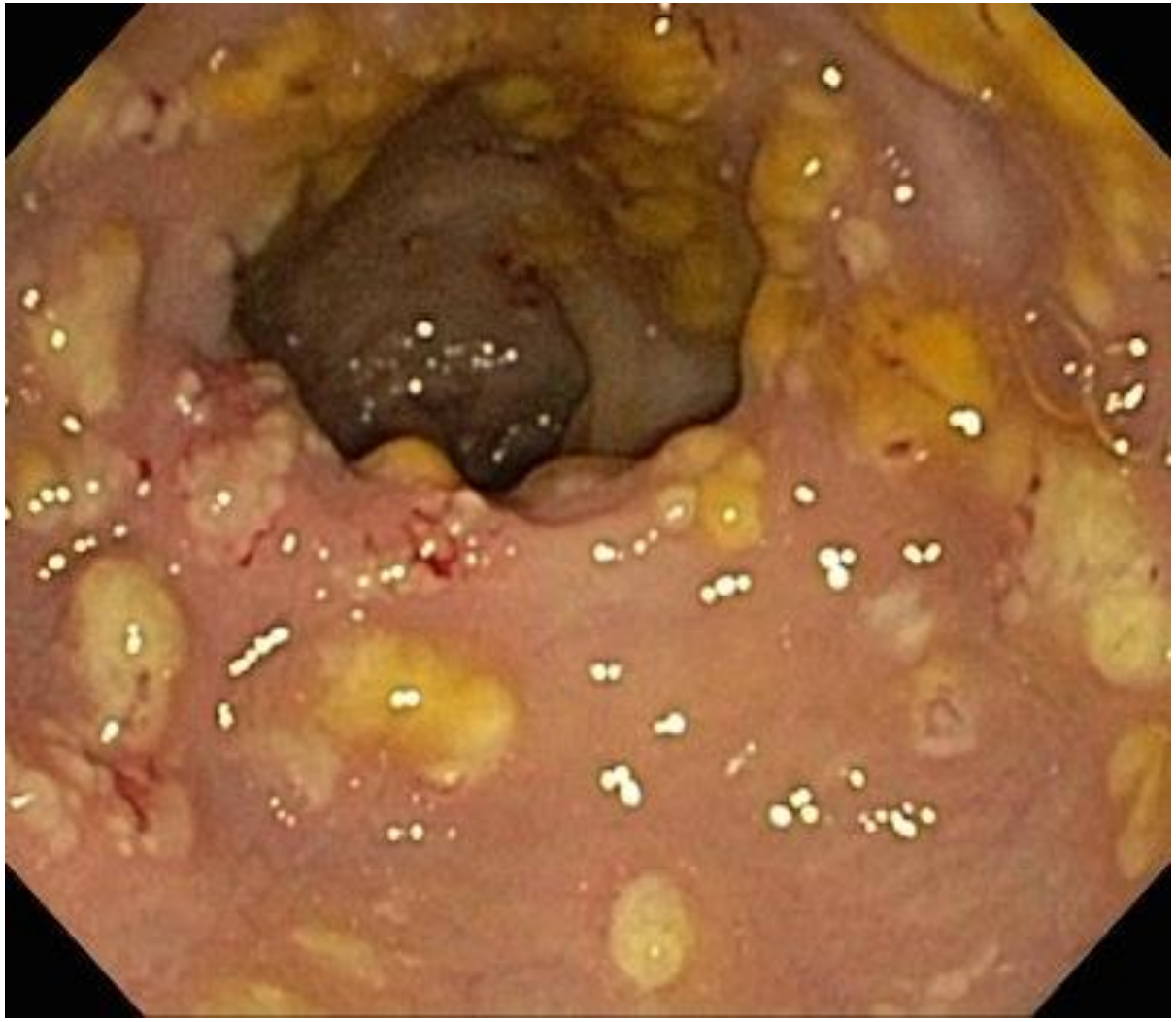
- ***C. difficile*** causes ***pseudomembranous enterocolitis*** clinically manifested by diarrhea. The disease occurs as a result of an imbalance between anaerobic bacteria - bacteroids and bifidobacteria, which play an important role in the formation of colonization resistance, which arose against the background of irrational antibiotic therapy.
- C.difficile produces two exotoxins.
- - ***A toxin*** has enterotoxic as well as cytotoxic effects. Binds to the intestinal epithelium through specific receptors for microvilli
- - ***B toxin*** - cytotoxic action.

Pseudomembranous colitis

- Pseudomembranous colitis is manifested by colicky abdominal pain, liquid or bloody diarrhea, leukocytosis and hyperthermia.
- Endoscopically, it is possible to detect necrotic remnants of the mucosa, fibrin, leukocytes that form a ***pseudomembrane*** in the affected part of the intestine.

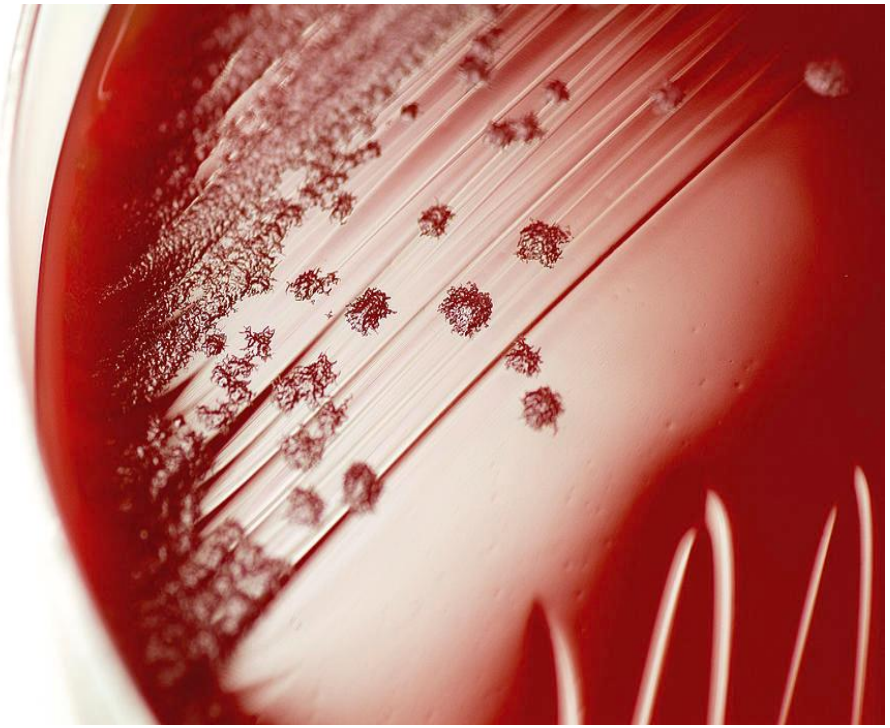
Pseudomembranous colitis

(endoscopy of the intestinal mucosa)



Microbiological diagnostics

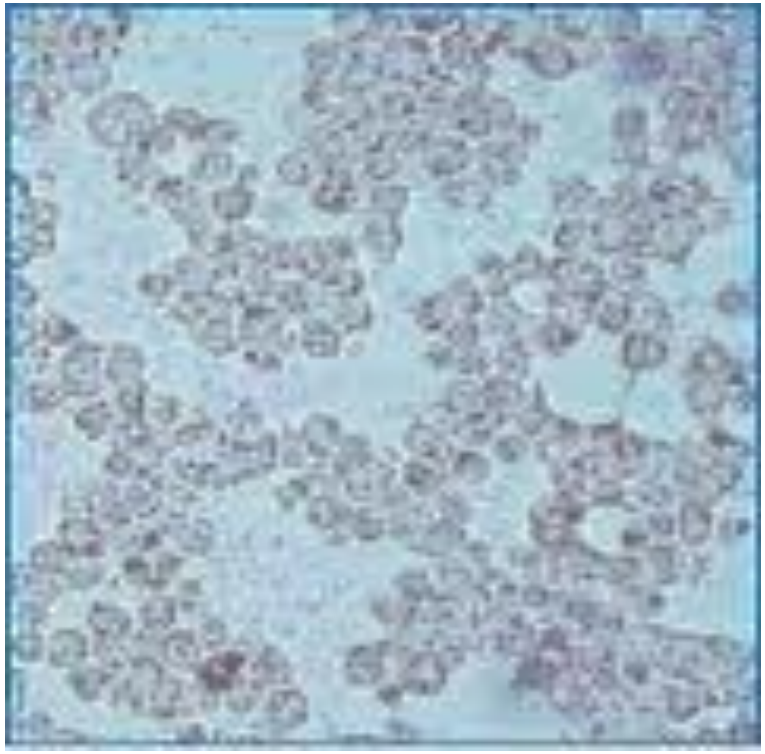
- ***The bacteriological method*** is based on the cultivation of material in selective nutrient media under anaerobic conditions. Sowing is done on yolk-fructose agar with the addition of cycloserine and cefoxitin (CCFA agar). Bacteria form opaque glassy yellow colonies.



Effect of C.difficile toxin on sensitive cells

(human embryonic fibroblasts)

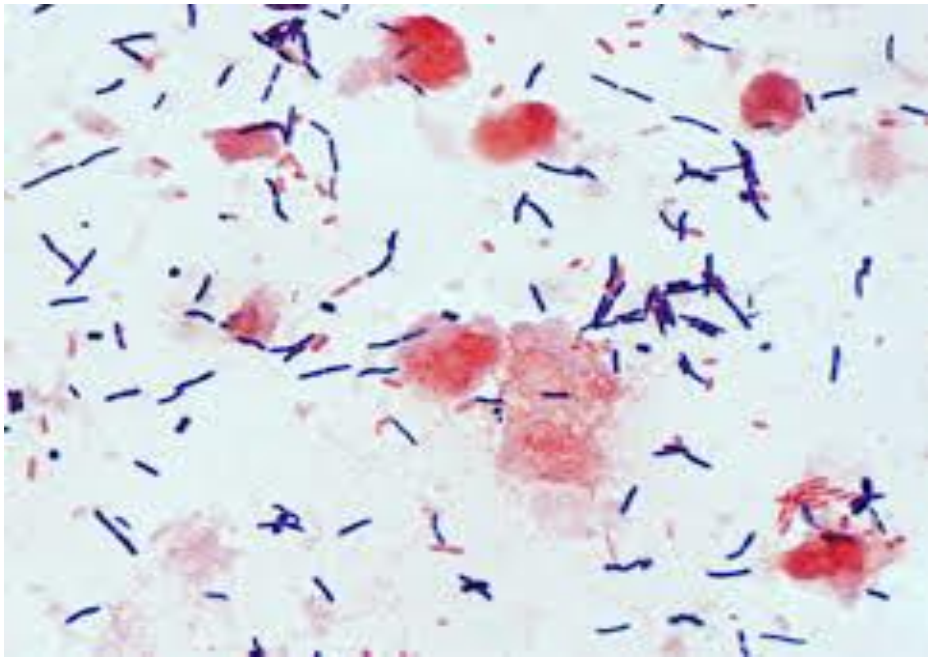
Detection of C.difficile toxins in feces is based on determining the effect on cell culture(embryonic fibroblasts) and is of great diagnostic value.



microtests for the detection of C.difficile toxin in feces.

Microbiological diagnostics

- Microscopic and bacteriological examination of feces.
- ***Microscopic examination*** reveals large gram-positive bacteria.



C.difficile (pure culture smear with Gram stain)

Treatment

- Vancomycin and metronidazole
- ***Enterol*** is a preparation containing yeast (Saccharomyces boulardii).

Morpho-biological properties:

Corynebacterium diphtheriae *are thin, slightly curved or straight Gram-positive polymorphic rods 1-1.2x0.3-0.8 μm in size. They are non-motile, do not have a capsule, do not form spores. They are thickened at the ends due to the presence of volutin grains (Babes-Ernst grains). Volutin grains are easily detected by Neisser staining as dark blue or blue-black granules, respectively.*

C. diphtheriae has four biovars:

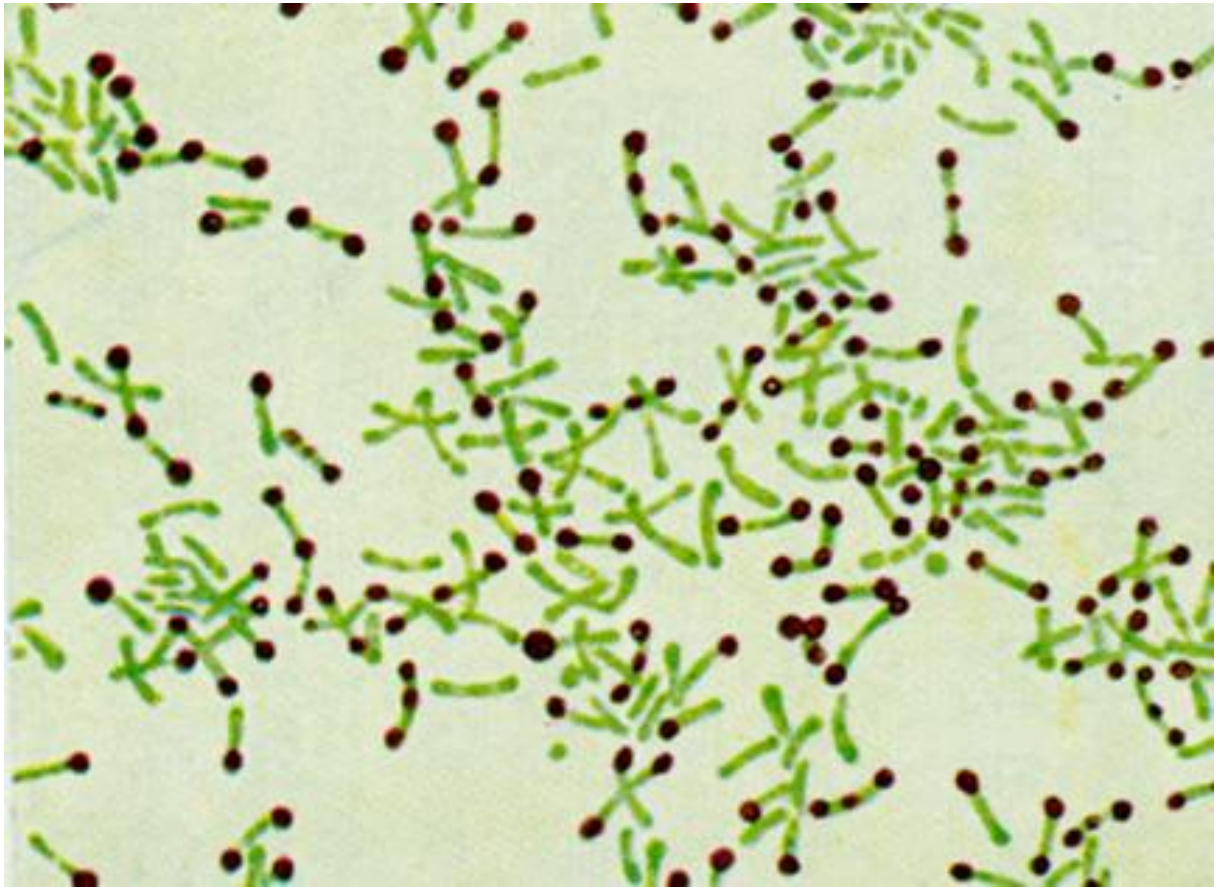
gravity

mitis

intermediate

belfanti

Corynebacterium diphtheriae



Volutin grains (in Neisser-stained smears)

Corynebacterium diphtheriae cultural properties:

- ***Clauberg's medium*** *(medium supplemented with blood serum and potassium tellurite)*
- ***Clauberg's medium II*** *(blood tellurite agar)*

A- biovar gravis
Dry matte greyish black R-colonies
"daisy flower"

B-biovar mitis
Smooth, shiny, black translucent
S-colonies

Corynebacterium diphtheriae cultural properties:

- *C. diphtheriae on tellurite media grow as black or black-gray colonies as a result of the reduction of tellurite to metallic tellurium.*

C. diphtheriae (colonies on tellurite medium)

Differential features of bacteria of the genus Corynebacterium

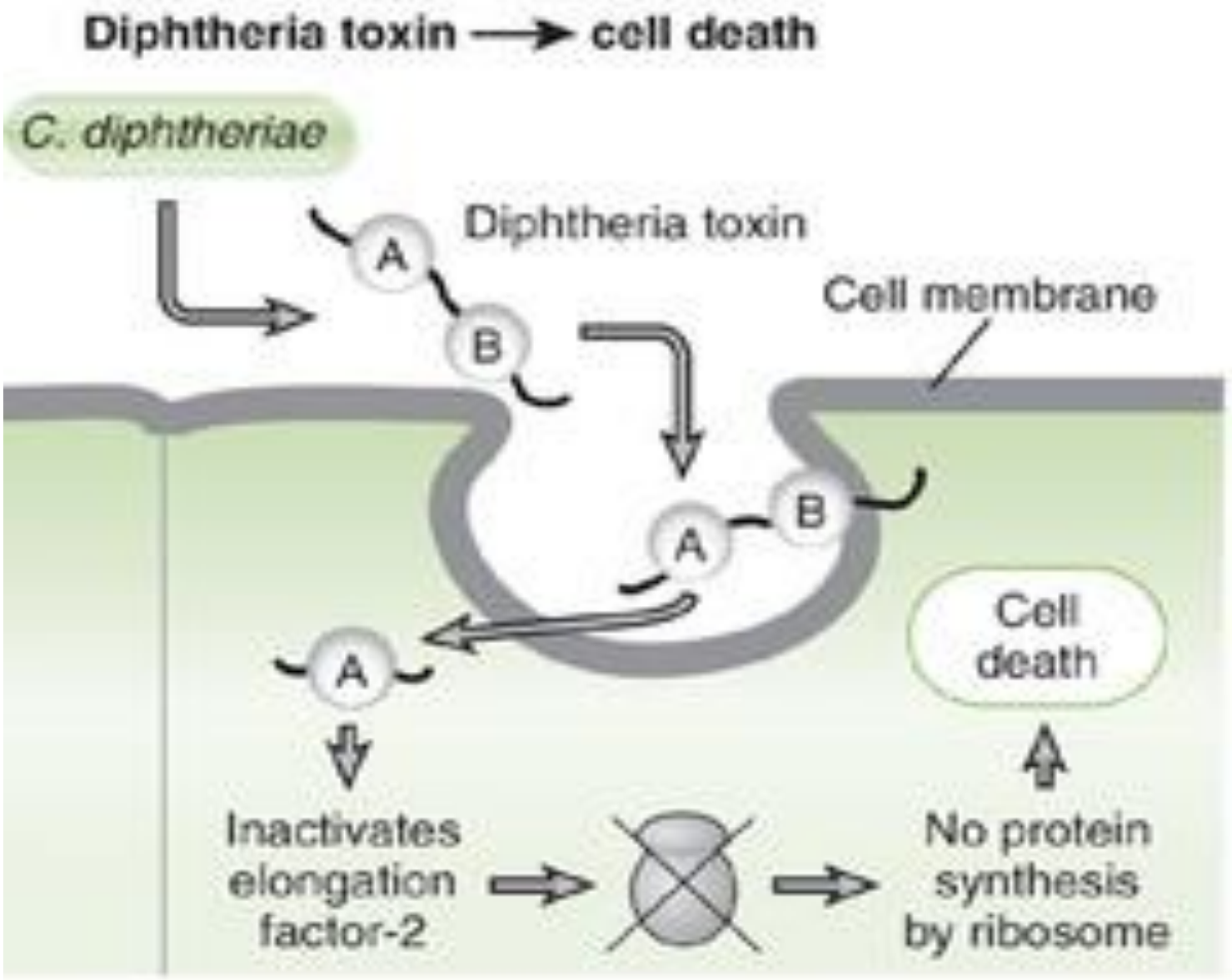
View	Ability to decompose:				Nitrate recovery
	cystine	Urea	Glucose	Starch	
C.diphtheriae gravis mitis intermedius belfanti	 + + + +	 - - - -	 + + + +	 + - - -	 + + + -
C.pseudodiphtheriae	-	+	-	-	+
C.xerosis	-	+	+	-	+
C.ulcerans	-	+	+	-	+
C.jeikeum	-	+	+	-	-
C.sistidis	-	+	+	+	-
C.minitissumum	-	+	+	-	-

Pathogenic factors:

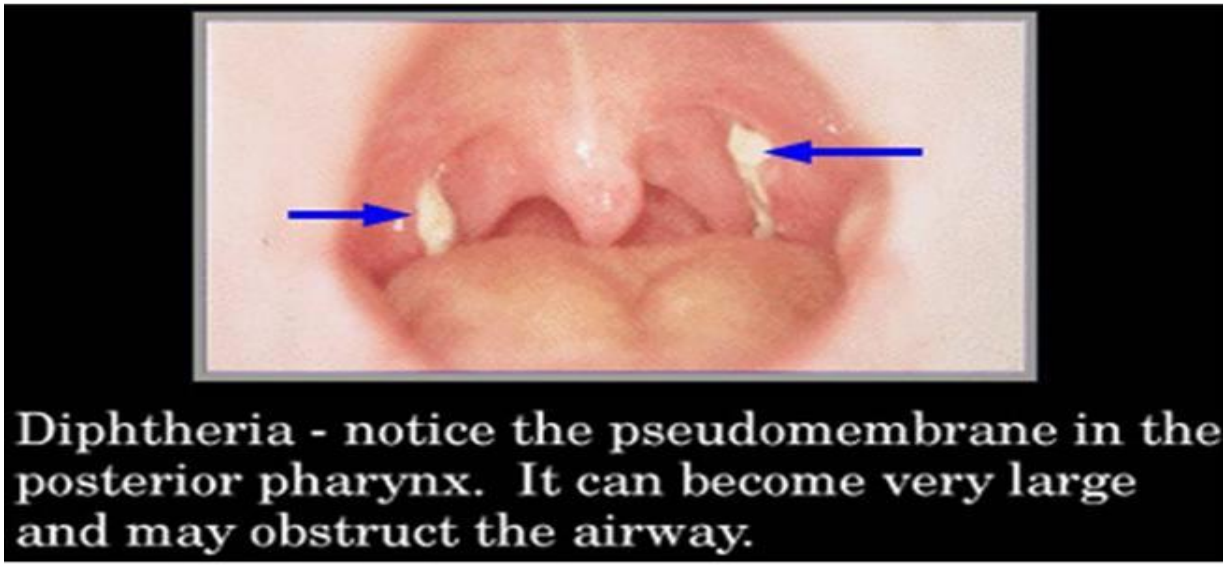
- ***Surface structures*** *(substances of a lipid and protein nature contribute to the adhesion of the microbe)*
- ***Fimbriae*** *(micropili)*
- ***Enzymes of aggression and invasion*** *(promote penetration into the blood and tissues)*

- ***hyaluronidase***
- ***neuraminidase***
- ***fibrinolysin***
- ***dermonecrotoxin***
- ***exotoxin (histotoxin)*** **-** *the main factor of pathogenicity*

Mechanism of action of C.diphtheriae exotoxin:



Diphtheria of the throat - **an inflammatory reaction develops in the area of the entrance gate of infection, accompanied by necrosis of epithelial cells, edema, fibrinogen release from the vascular bed and its transformation into fibrin. This leads to the formation of a white plaque with a grayish tint, containing a large number of bacteria producing exotoxin - fibrinous film. The film is hardly removed with a swab and when you try to remove it, the mucous membrane bleeds.**



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

Microbiological diagnostics:

- ***Materials for research:***

Detachable pharynx

Nasal discharge

Detachable from suspicious lesions of the skin The sampling of the material is carried out using sterile Dacron swabs.

- ***Microscopic method*** **- in smears stained with methylene blue or Gram, diphtheria bacilli are not always morphologically typical (polymorphism of bacteria makes identification difficult).**

Microbiological diagnostics

- ***Bacteriological method*** - carried out in order to isolate a pure culture of C. diphtheriae and identify it by morphological, cultural, biochemical and toxigenic properties. To do this, the pathological material is seeded on blood agar, Loeffler's medium or medium with tellurite.
- ***The agar precipitation reaction is based on the ability of diphtheria exotoxin to interact with antitoxic antibodies.***

Determination of C. diphtheria toxigenicity

Elek Method– a strip of filter paper impregnated with diphtheria antitoxin is placed on a Petri dish with phosphate-peptone agar, and the studied cultures are inoculated with plaques on both sides of the strip at a distance of 7-9 mm. After 48 hours of incubation, as a result of counter-diffusion of toxin and antitoxin, a precipitation line is formed at the place of their contact.

Elek's test positive

Microbiological diagnostics

- **Polymerase chain reaction - detection of the diphtheria bacillus gene in pathological material**
- **Enzyme-linked immunosorbent assay (ELISA) - used to detect C. diphtheriae toxin in clinical material.**
- **Immunochromatographic method - an express method used to detect diphtheria toxin from pathological material using special test strips, panels or test cassettes.**

Specific prophylaxis

- ***DTP vaccine*** - (adsorbed pertussis-diphtheria-tetanus vaccine)
- ***ADS toxoid*** (adsorbed diphtheria-tetanus toxoid)
- ***ADS-M-toxoid*** (adsorbed diphtheria-tetanus toxoid with a reduced content of antigens)
- ***AD-M-toxoid*** (adsorbed diphtheria toxoid with reduced antigen content)
- Vaccination is carried out from the age of three months.

Treatment

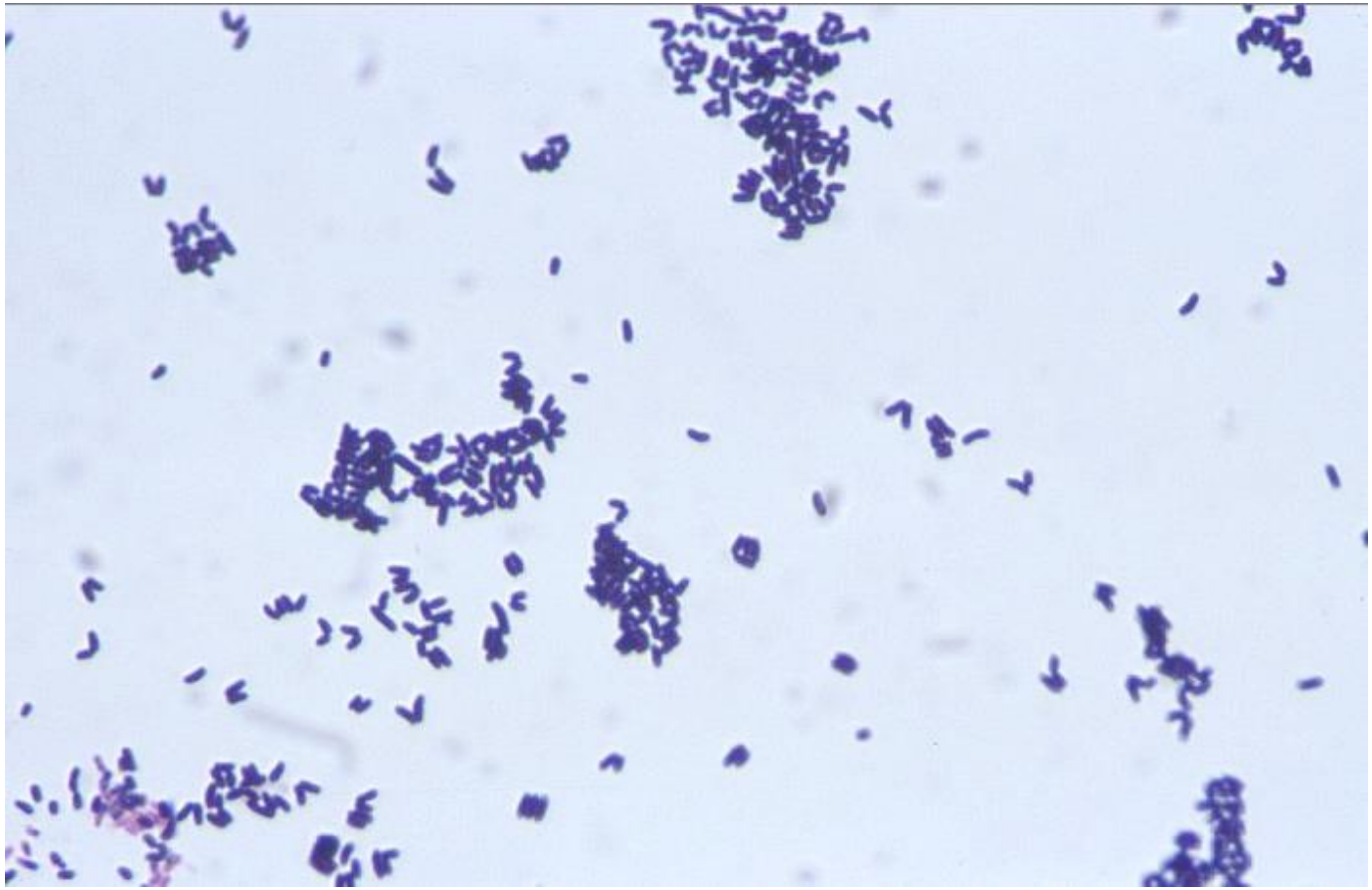
- Treatment is started immediately with clinical suspicion of diphtheria, since the duration of laboratory tests is unacceptably dangerous!
- ***The basis of specific therapy is antidiphtheria serum.***
- ***Antibiotics***

  penicillin

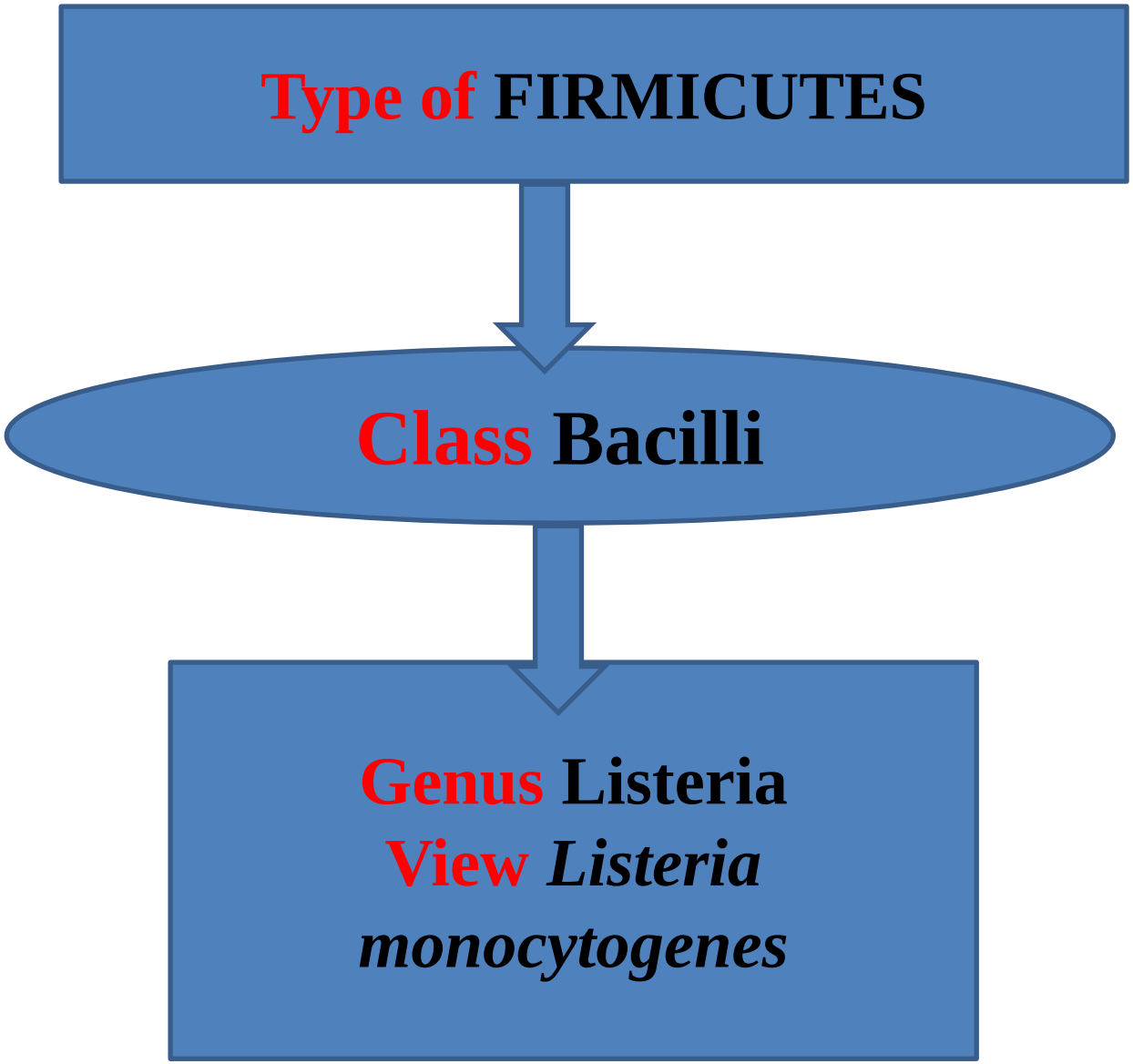
  beta-lactam antibiotics

  macrolides

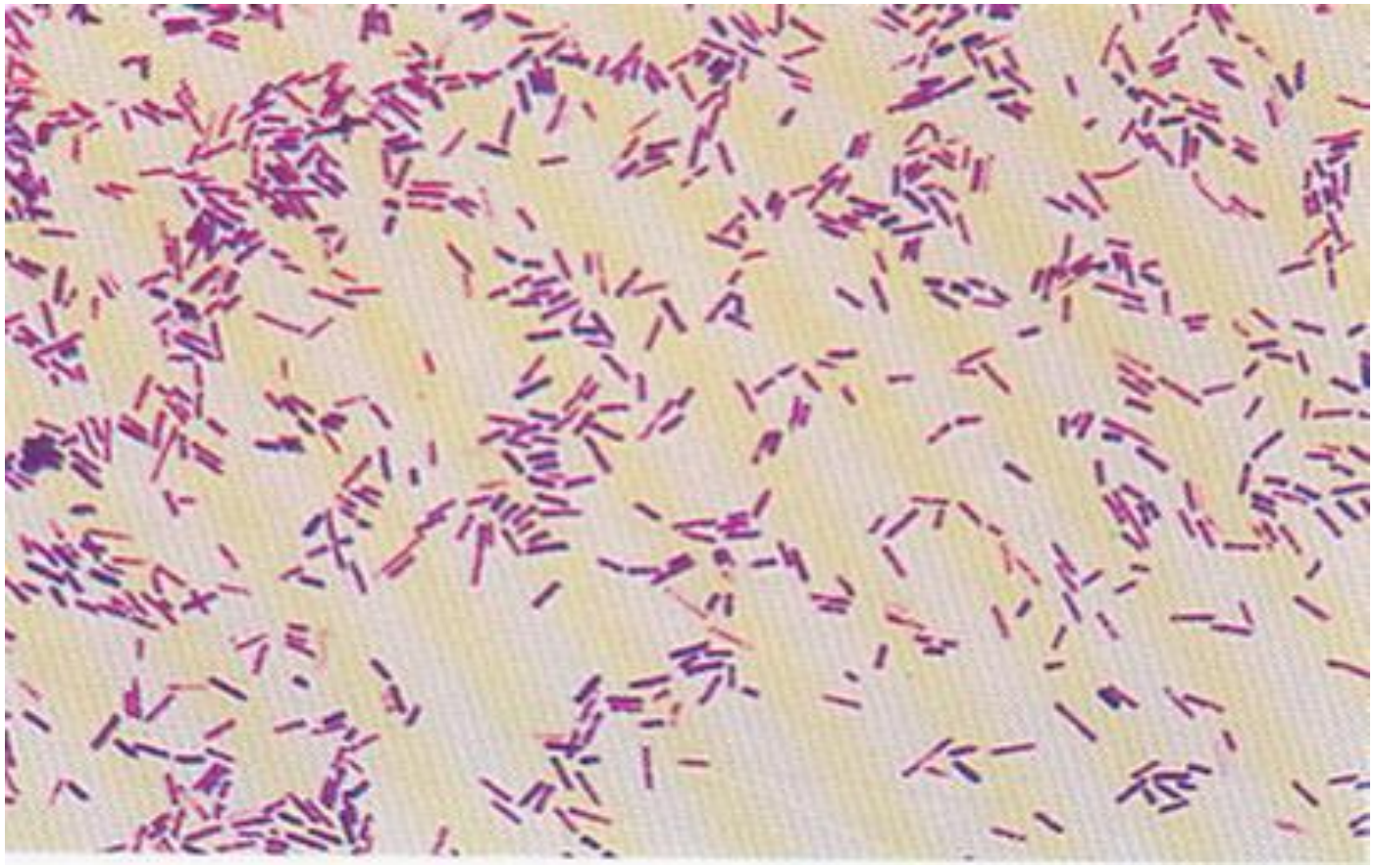
Diphtheroids are a group of opportunistic bacteria of the genus *Corynebacterium, referred to as coryneform bacteria. Diphtheroids* are located in micropreparations in the form of a uniform palisade, do not have volutin grains or they are located on one of the poles.



diphtheroids (pure culture swab)


Type of FIRMICUTES
Class Bacilli
Genus Listeria
View Listeria monocytogenes

L. monocytogenes **are motile Gram(+) rods. In strokes, they are arranged at an angle to each other, resembling hieroglyphs. Can form a capsule**

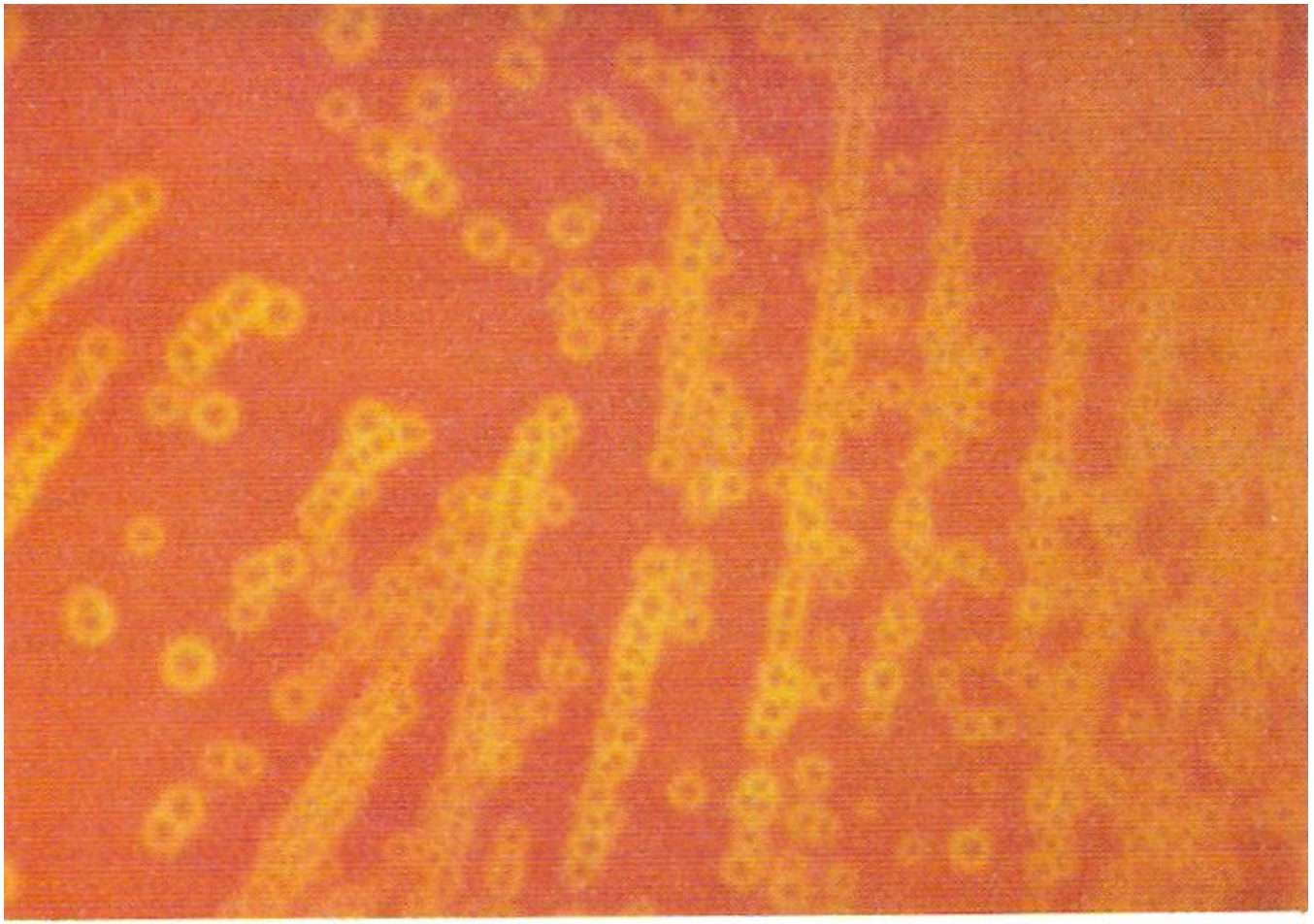


Cultural and biochemical properties

- **Catalase**-positive
- Microaerophiles, grow at 5% CO2
- Cause hemolysis on CA. They can produce a yellow or reddish pigment.
- Ferment glucose and other carbohydrates to acid
- They have O and H antigens.

Listeria monocytogenes

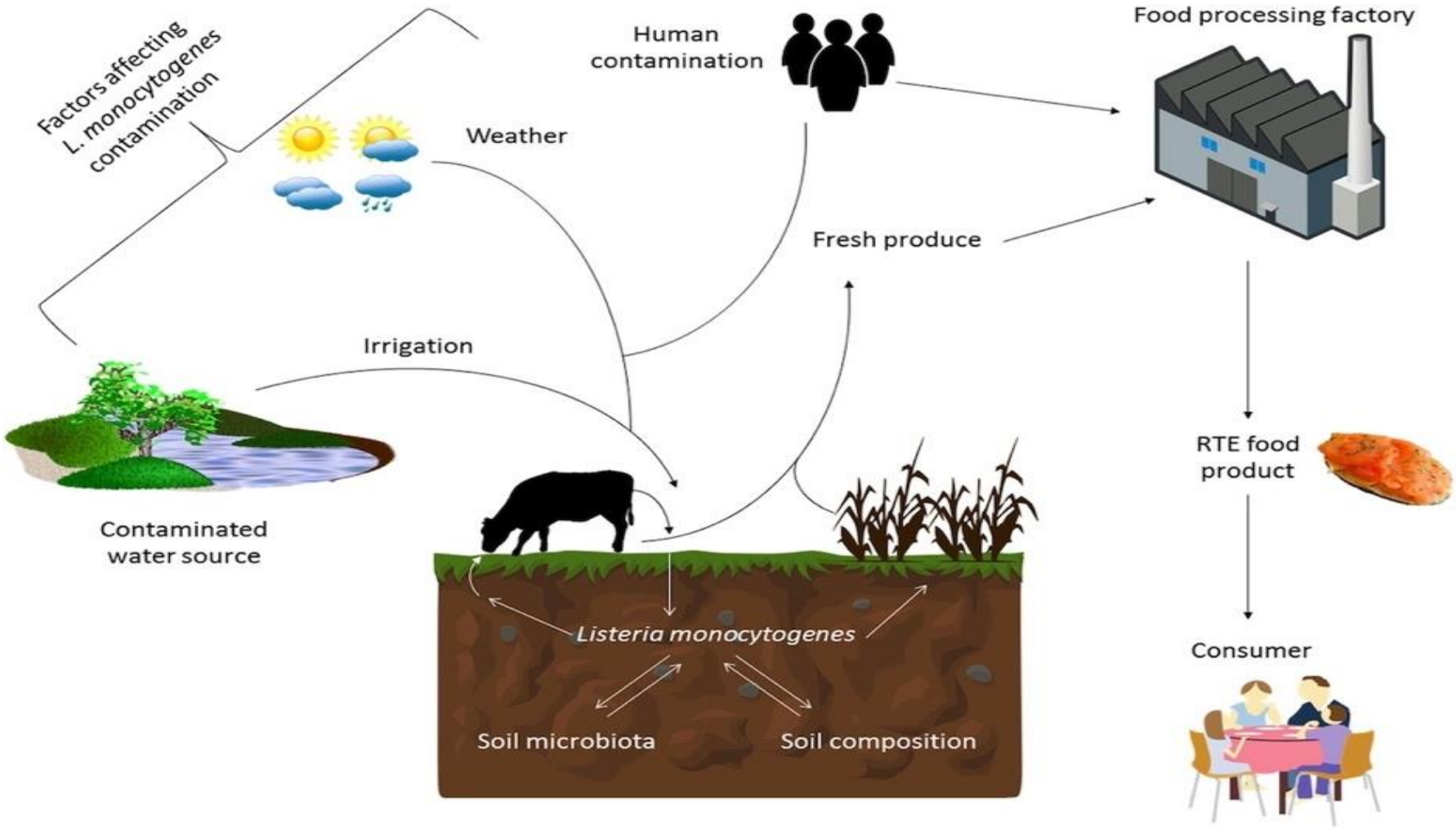
blood agar culture



Pathogenicity factors

- ***Internalin and extracellular protein*** - facilitate penetration into cells
- ***Listeriolysin O -*** hemolysis of erythrocytes, damage to the phagosome membrane
- ***Phospholipase C -*** destruction of cell membranes, phagosome lysis, distribution throughout the body
- ***Act A*** is an actin polymerizing protein that ensures the movement of bacteria through the cytoplasm and between cells.
- ***Lecithinase*** promotes intracellular survival and reproduction

Listeriosis infection



Epidemiology and pathogenesis of listeriosis

- The source of infection is the soil, where the microbe exists freely and infects the roots of plants, in particular vegetables (sopronosis).
- Secondary source - wild (boars, foxes, hares) and domestic (sheep, pigs, cattle, cats) animals
- Listeria tolerate low temperatures well. At a temperature of 4°C, they are able to multiply in dairy and meat products.
- The main route of infection is alimentary, less often - contact, aerosol and vertical.
- In pathogenesis, the state of immunosuppression is important. From the entrance gate, the pathogen disseminates lymphogenously and hematogenously into the internal organs, causing the formation of pnecrotic nodules - listeriomas.
- There are acquired and congenital listeriosis.

Патогенез листериоза

LOGO

Входные ворота

- Слизистые оболочки ЖКТ
- Слизистые репираторного тракта и глаз
- Поврежденные кожные покровы

При лимфогенном и гематогенном распространении происходит фиксация листерий в лимфатических узлах и внутренних органах – миндалинах, легких, в печени, в селезенке, почках, надпочечниках, ЦНС.

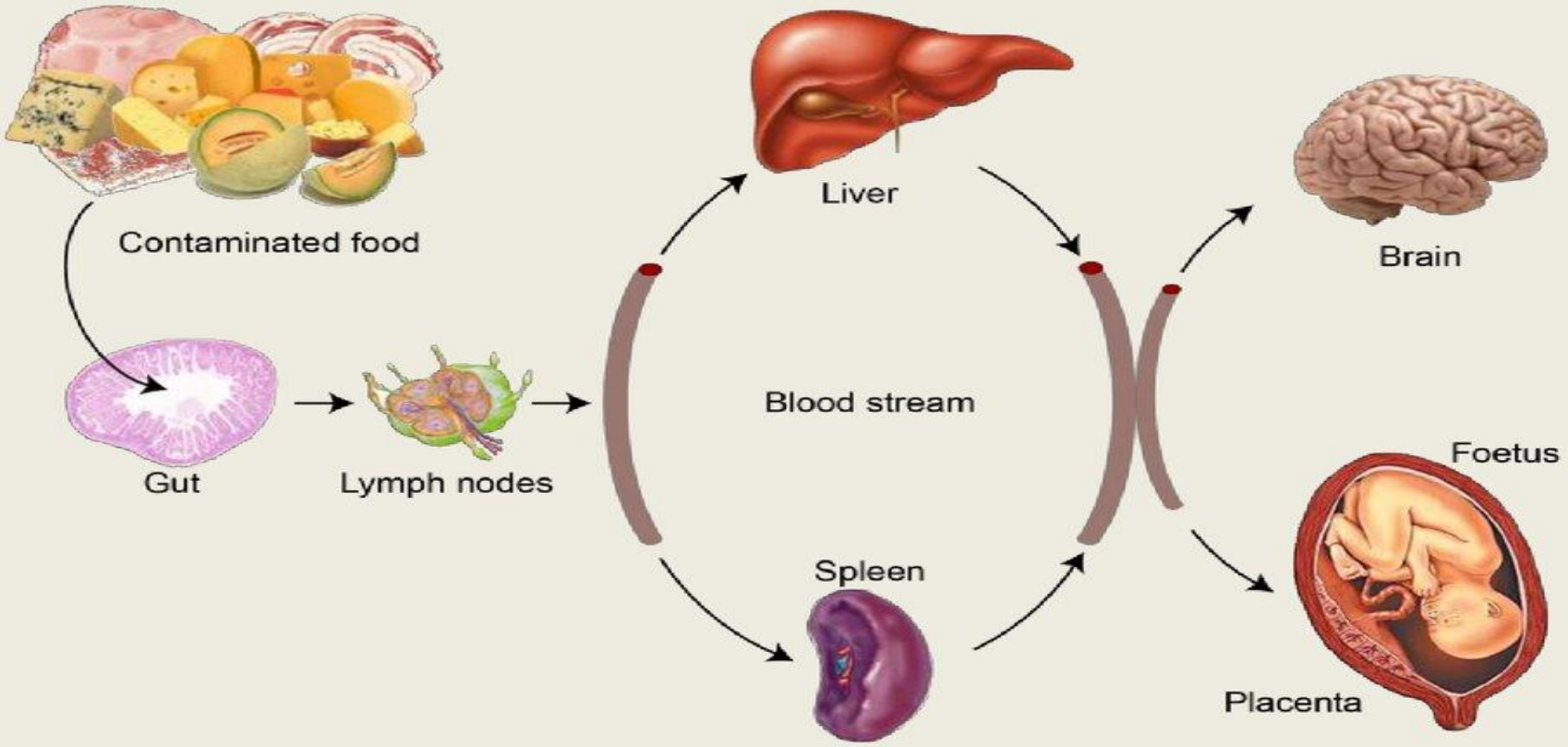
При беременности некротические узелки (листериомы) могут формироваться в плаценте, что приводит к инфицированию плода, с развитием у него генерализованной формы инфекции.

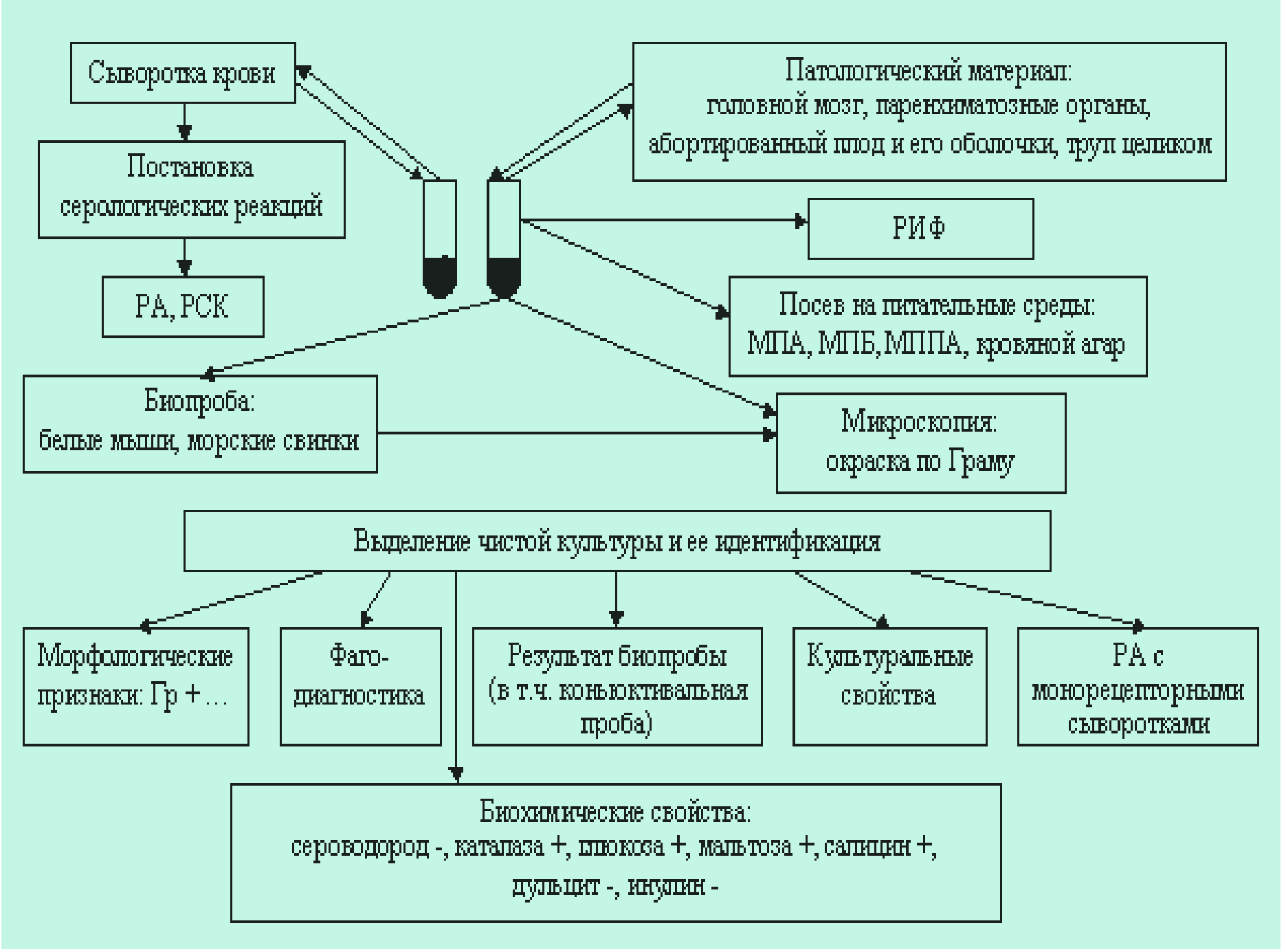
Заболевание способствует иммунодефецитным состояниям и опухолям.

Causes meningitis and sepsis in immunocompromised individuals
Causes fever and sepsis in pregnant women

Sepsis
Granulomatosis
Meningitis
In newborns

ПАТОГЕНЕЗ




Сыворотка крови
Постановка серологических реакций
РА, РСК
Патологический материал: головной мозг, паренхиматозные органы, абортированный плод и его оболочки, труп целиком
РИФ
Посев на питательные среды: МПА, МПБ, МППА, кровяной агар
Биопроба: белые мыши, морские свинки
Микроскопия: окраска по Граму
Выделение чистой культуры и ее идентификация
Морфологические признаки: Гр +…
Фаго-диагностика
Результат биопробы (в т.ч. конъюктивальная проба)
Культуральные свойства
РА с монорецепторными сыворотками
Биохимические свойства: сероводород -, каталаза +, глюкоза +, мальтоза +, салицин +, дульцит -, инулин -