Lesson XIV

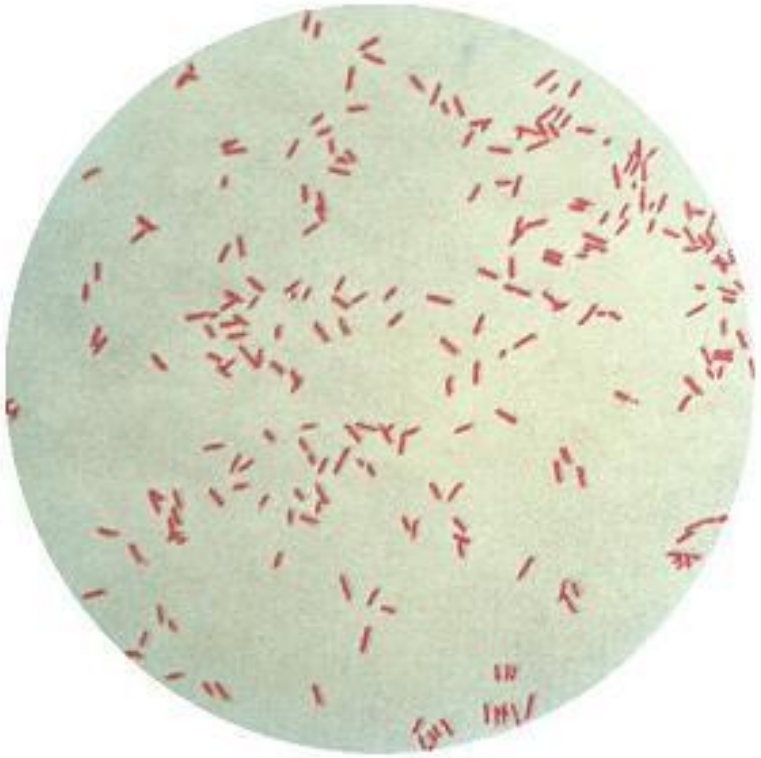
Microbiological diagnosis of infections caused by gram-negative rods (genera Pseudomonas, Bacteroides, Legionella, Haemophilus, Bordetella)

Genus Pseudomonas (taxonomy)

- *More recently, some bacteria of the genus Pseudomonas have been assigned to the genus Burkholderia.*
- *Representative of the genus Pseudomonas Pseudomonas aeruginosa (Pseudomonas aeruginosa) is the causative agent of many pyoinflammatory diseases*
- *Representatives of the genus Burkholderia: Burkholderia (Pseudomonas) mallei - the causative agent of glanders and Burkholderia (Pseudomonas) pseudomallei - the causative agent of melioidosis.*

Pseudomonas aeruginosa -

Gram-negative, motile rods, do not form spores

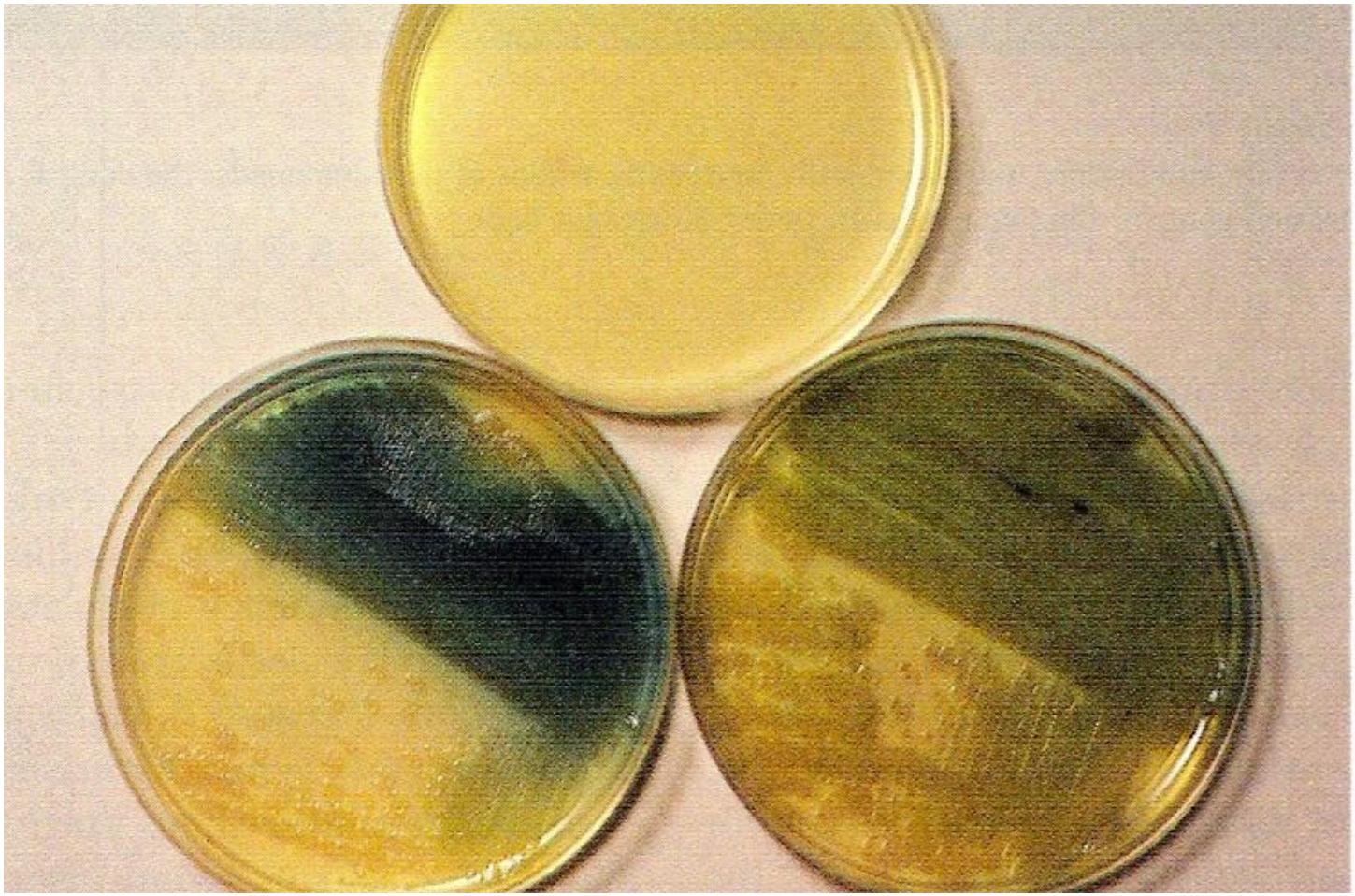


Pseudomonas aeruginosa

(growth on nutrient media)

- On blood agar: ß-hemolysis
- On Endo and McConkey media: lactose-negative colonies (do not ferment lactose)
- On meat peptone agar: cloudy colonies with jasmine odor

Pseudomonas aeruginosa culture



Pseudomonas aeruginosa

(pathogenicity factors)

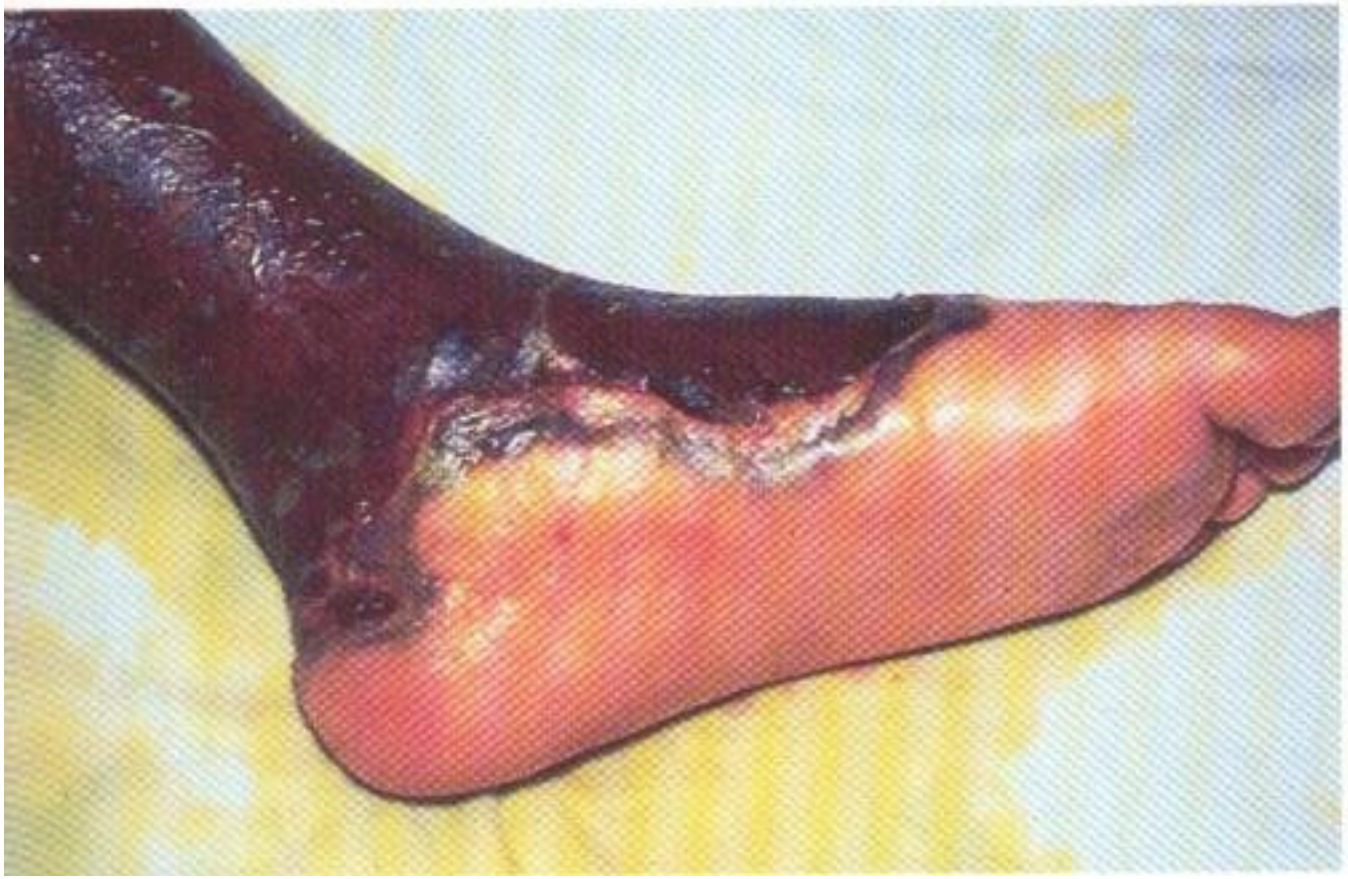
- drinking
- Extracellular mucus
- Toxins:
- LPS
- Exotoxin A
- Exotoxin S
- Leukocidin
- Enterotoxin
- Enzymes of aggression:
- Hemolysin
- Neuraminidase
- Protease
- Elastase

Role *Pseudomonas aeruginosa*

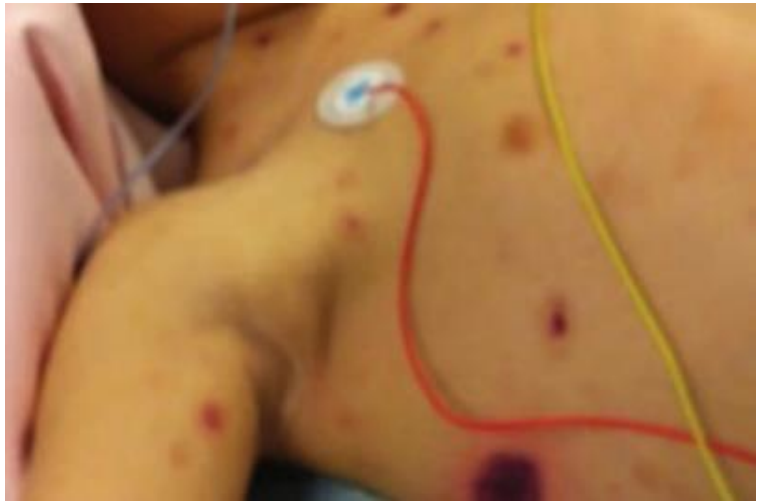
in human pathology

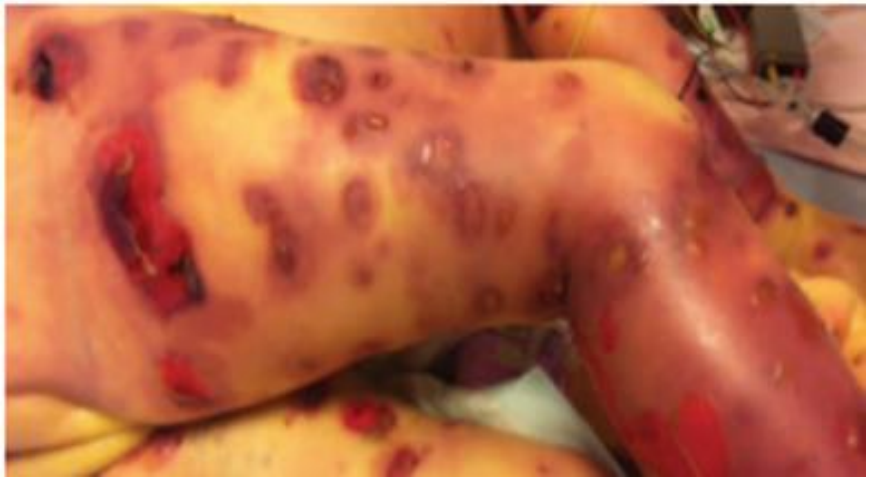
- burn disease
- Purulent infections of surgical wounds
- Keratitis
- Otitis
- cystic fibrosis
- Urinary tract infections
- Septicopyemia ("ecthyma gangrenosum")

Manifestations of Pseudomonas aeruginosa infection on the burn surface



Septicopyemia due to Pseudomonas aeruginosa ("ecthyma gangrenosum")





Microbiological diagnostics

Materials for research:

- Blood (for sepsis)
- Pus and wound discharge
- Urine
- Sputum

Microbiological diagnostics

Research methods

:

- ***Bacteriological (cultural)***
- ***Inoculation of the test material on simple and lactose-containing differential nutrient media***
- ***Incubation at a temperature of 37 for 18-24 hours***
- ***Identification by morpho-biological properties***
- ***Determination of sensitivity to antibiotics***

Non-spore forming anaerobic bacteria:

Gram-negative anaerobic bacteria:

- *Bacteroides*
- *Prevotella*
- *Porphyromonas*
- *Fusobacterium*
- *Leptotrichia*
- *Mobilincus*

Bacteroides (genus Bacteroides)

- *Member of the Bacteroideaceae family*
- *They are representatives of the normal microflora of the oral mucosa, upper respiratory tract, intestines and genital organs.*

Principles of classification of bacterioids

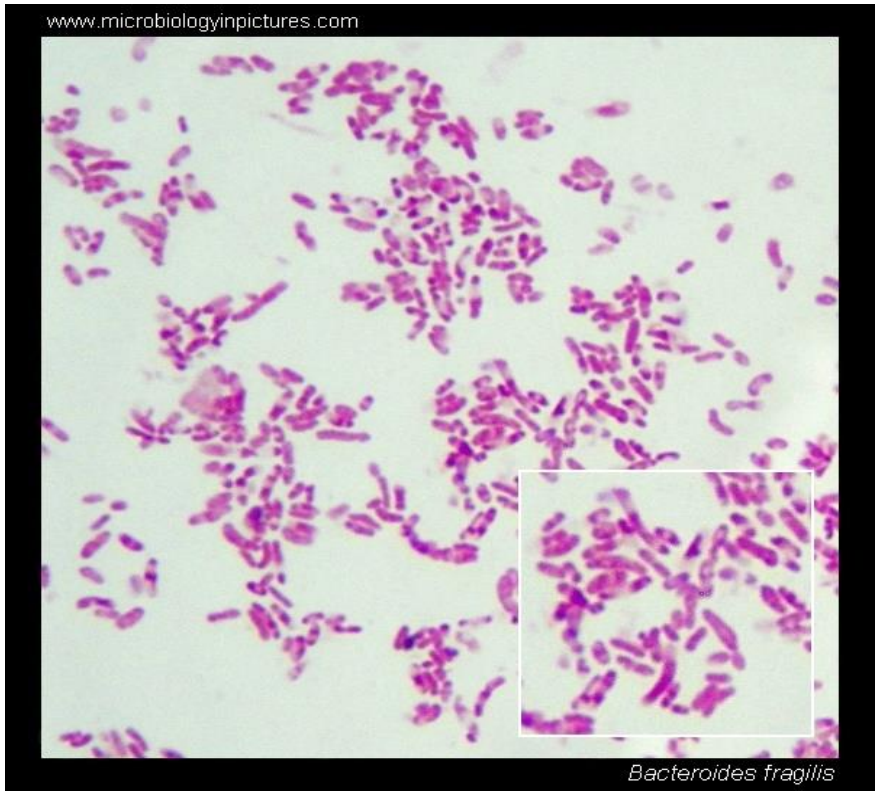
- Growth on media containing bile salts
- Pigment formation
- Sensitivity to antibiotics (kanamycin, vancomycin and colistin)
- ***Bacteria of the B.fragilis group are resistant to bile salts, do not form pigment, and are resistant to kanamycin, vancomycin and colistin.***
- ***Bacteroides that do not belong to the B.fragilis group are sensitive to bile salts, are characterized by the formation of pigments or the absence of this feature***

Bacteria of the B.fragilis group

- Bacteria of the genus Bacteroides (B.thetaiotaomicron, B.ovatus, B.vulgatus, B.distasonis B.uniformis, B.caccae, B.merdea, B.stercoris, B.ureolyticus, B.gracilis) similar to B.fragilis in terms of morpho-biological properties and ecological niche are included in the B.fragilis group.
- Bacteria of this group are part of the normal obligate microflora of the large intestine.

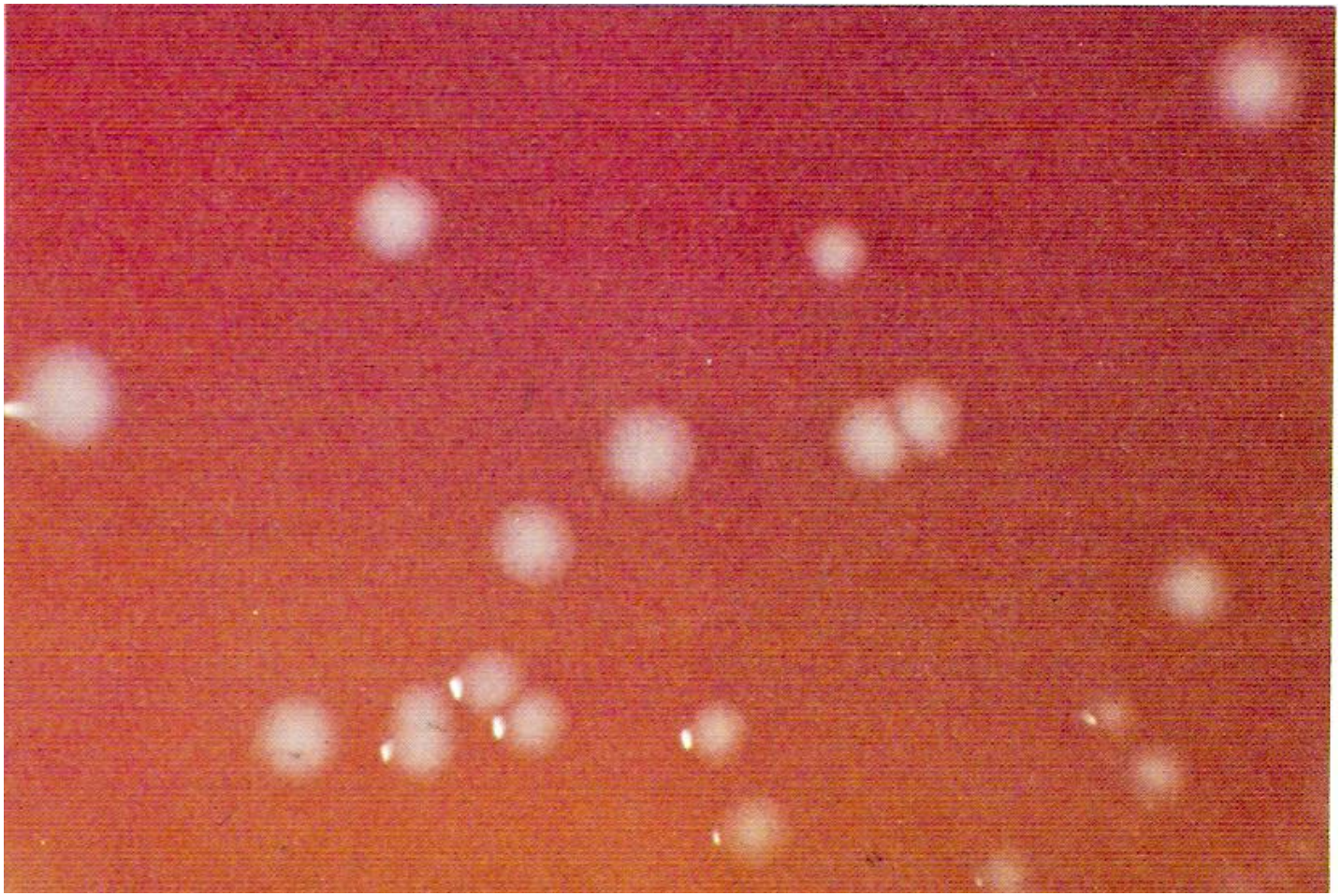
Bacteria of the B.fragilis group

- The bacteria of the B.fragilis group in Gram-stained smears from clinical material are represented by pale polymorphic rods with rounded ends or coccobacilli.
- Often stained unevenly by Gram due to intracellular vacuoles.



B.fragilis

(colonies on blood agar)



Pathogenicity of bacteroids

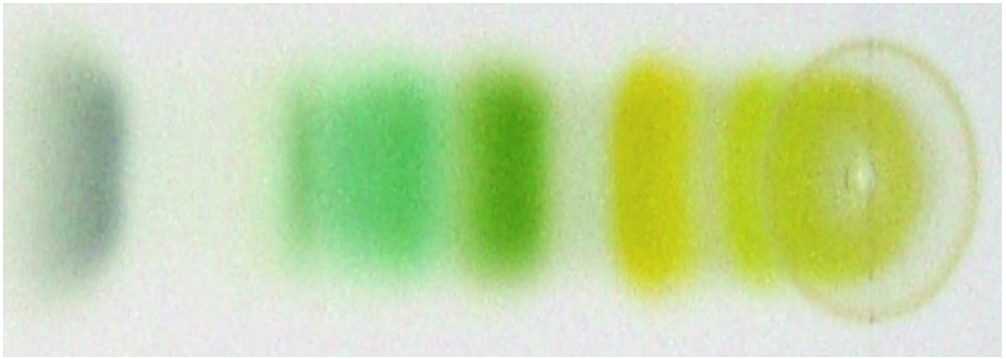
- Due to the fact that bacteroids belong to the normal human microflora, the vast majority of anaerobic infections caused by them are endogenous.
- With a decrease in the body's resistance, as well as in violation of the integrity of the mucous membrane, the bacteria translocate through tissue barriers and cause purulent-septic processes, most often abscesses.

Microbiological diagnostics

- In order to isolate a pure culture of bacteria, inoculations are carried out on appropriate nutrient media and cultivation under anaerobic conditions. Identification of isolated pure cultures is carried out on the basis of a study of morphological, cultural, tinctorial properties and enzymatic activity.
- Failure to grow on media containing bile salts, pigment formation, sensitivity to kanamycin, vancomycin and colistin are key diagnostic features

Microbiological diagnostics

- ***For express diagnosis of anaerobic infection, the gas-liquid chromatography (GLC) method is used.*** GLC is based on the chromatographic determination of volatile fatty acids, metabolic markers of obligate anaerobic bacteria. The detection of one or more volatile fatty acids, especially branched carbon isoacids, in the test material is evidence of the presence of obligate anaerobic bacteria.

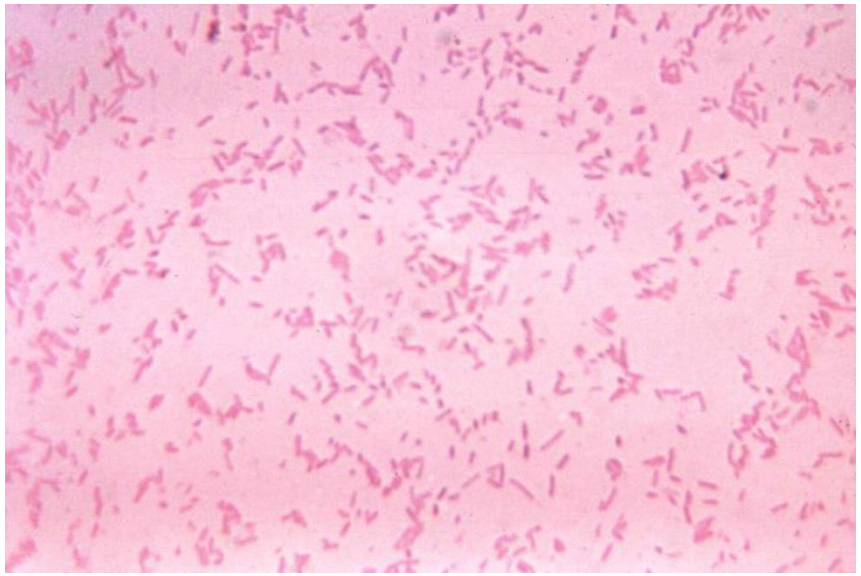


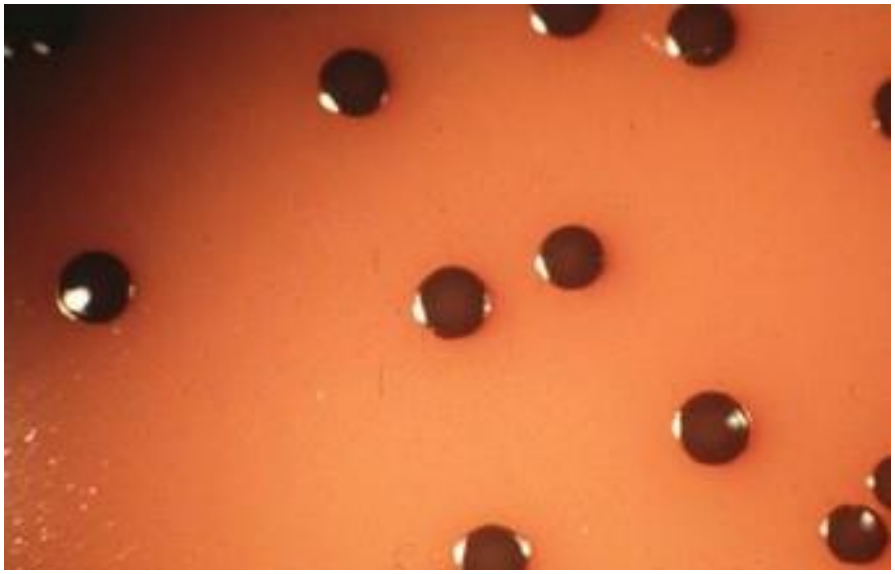
Gas liquid chromatography (GLC)

Gas liquid chromatography (GLC)

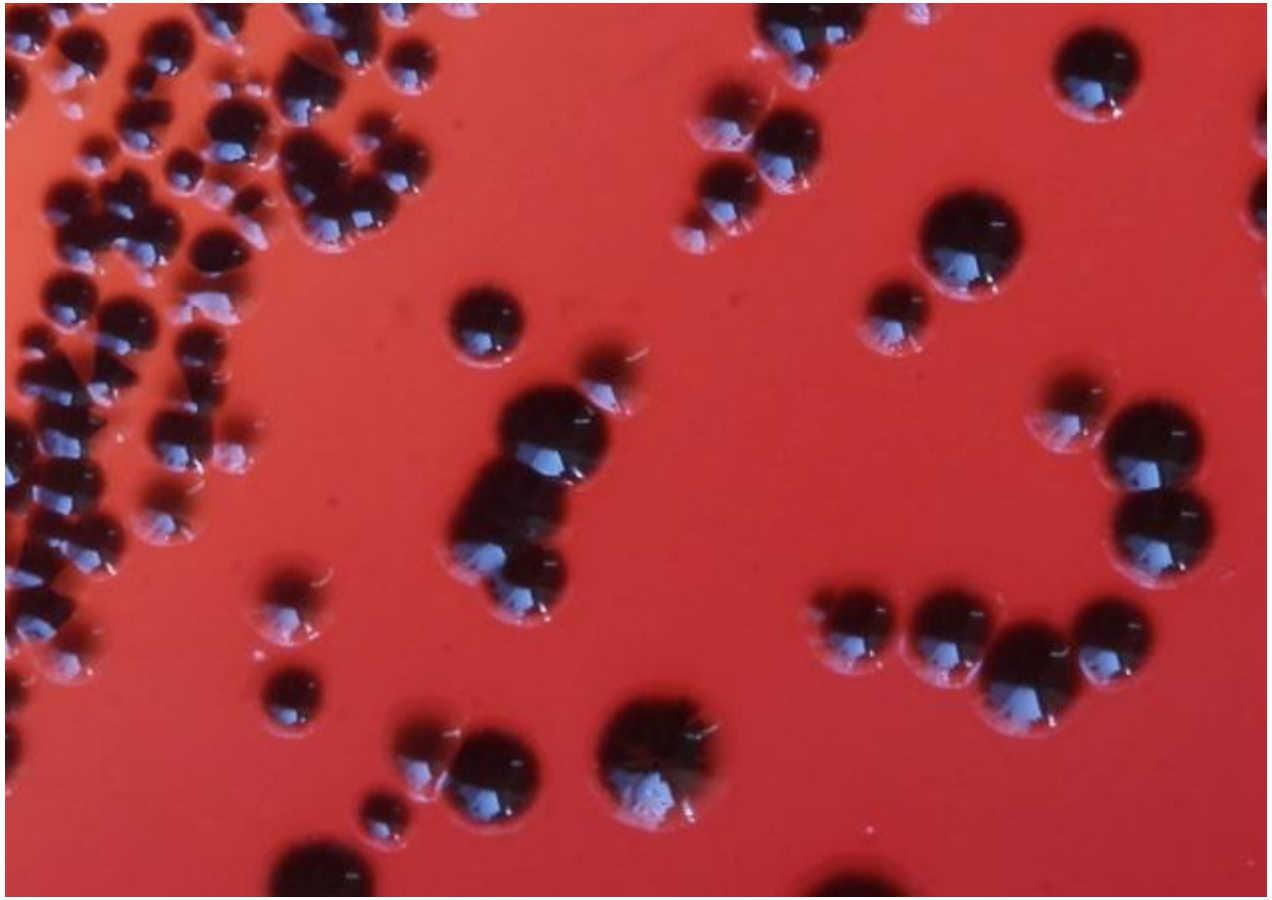
- imipenem
- clindamycin
- chloramphenicol
- nitroimidazole derivatives - metronidazole, tinidazole, ornidazole

Prevotella melaninogenica

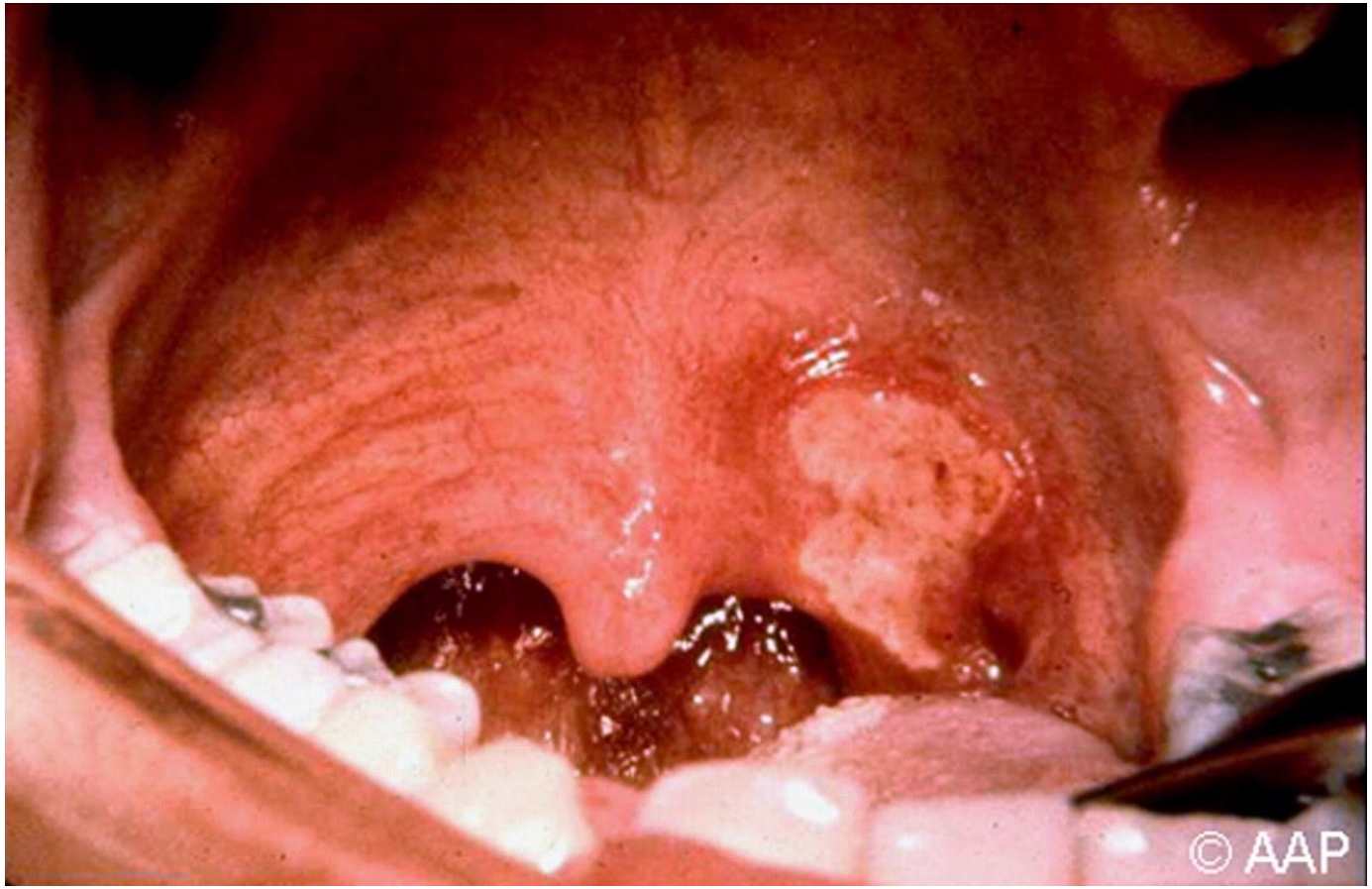




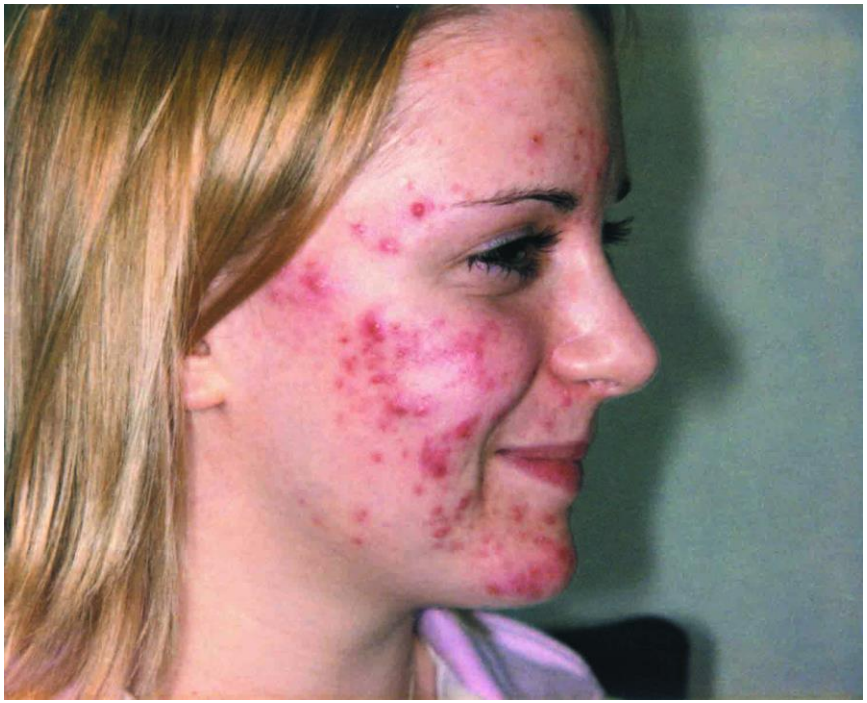
Colonies Porphyromonas gingivalis



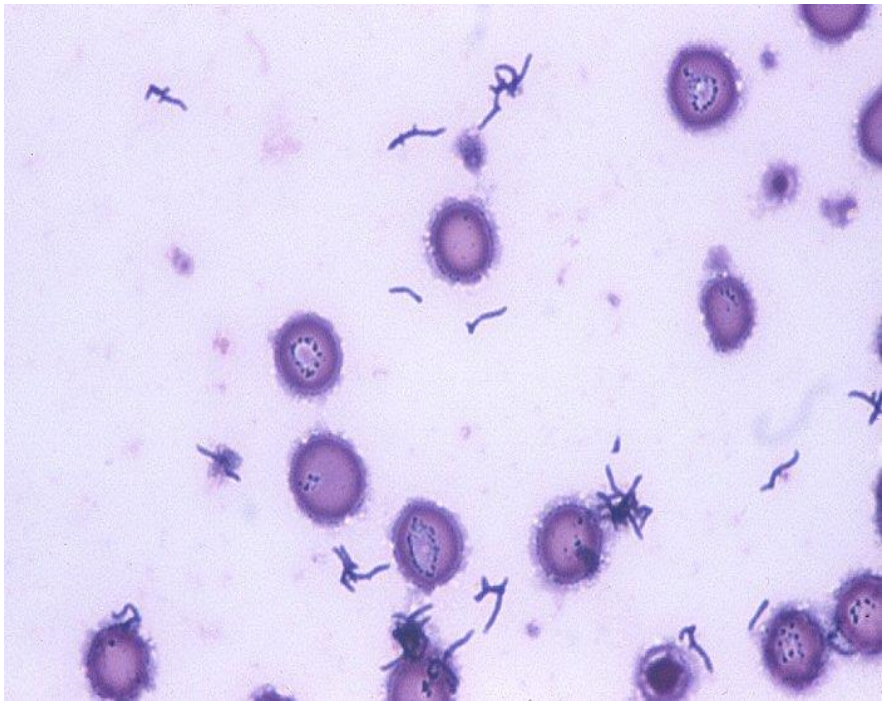
Angina Vincent - exudative pharyngitis, caused by Fusobacterium necrophorum in association with other anaerobic microflora of the oral cavity. A diphtheria-like plaque forms on the surface of the palatine tonsils.



Propionobacterium acnes– part of the normal microflora of the skin. Participates in the breakdown of free fats to fatty acids, which cause an inflammatory process - acne (pimples)

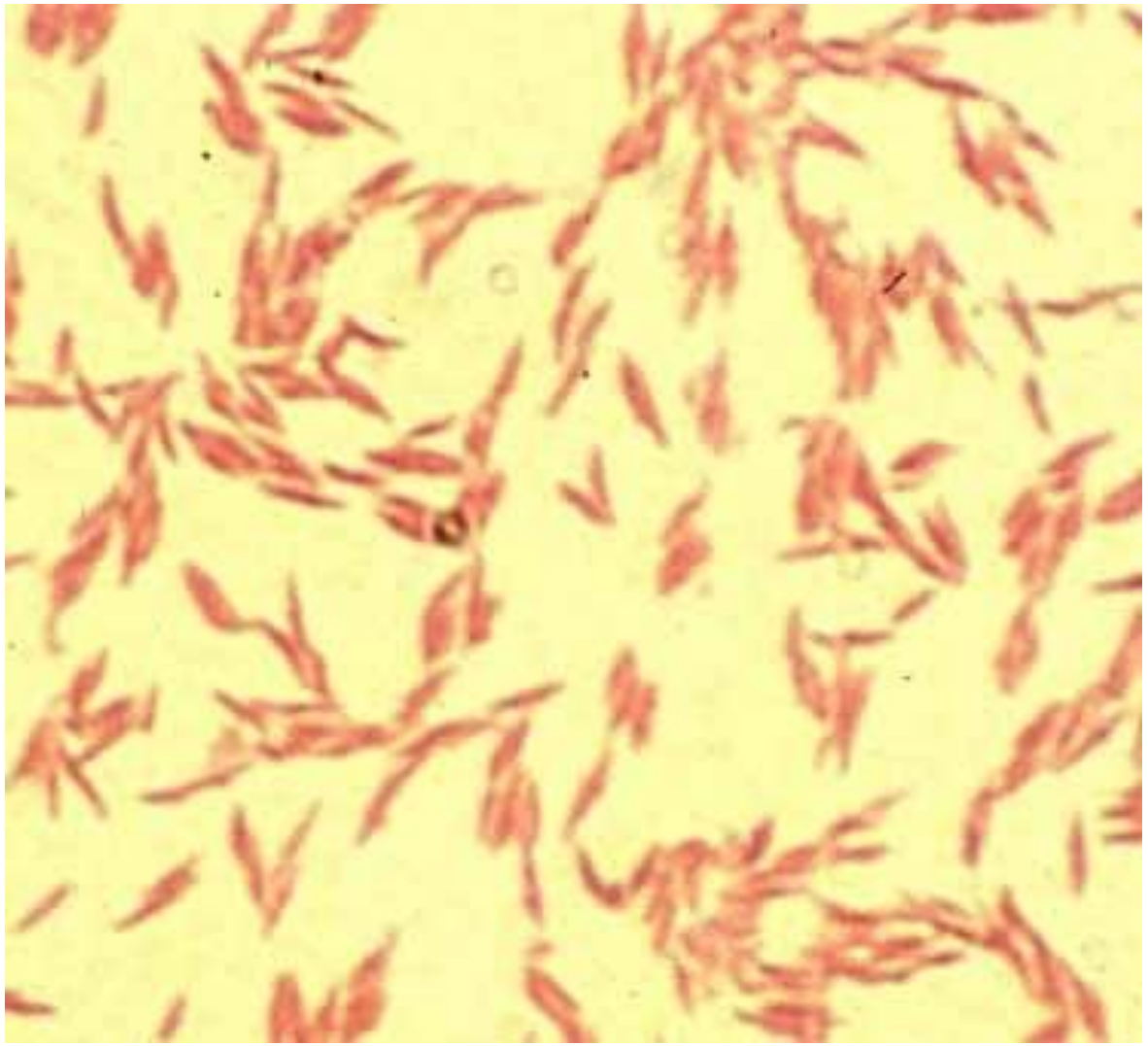


Acne (pimples)

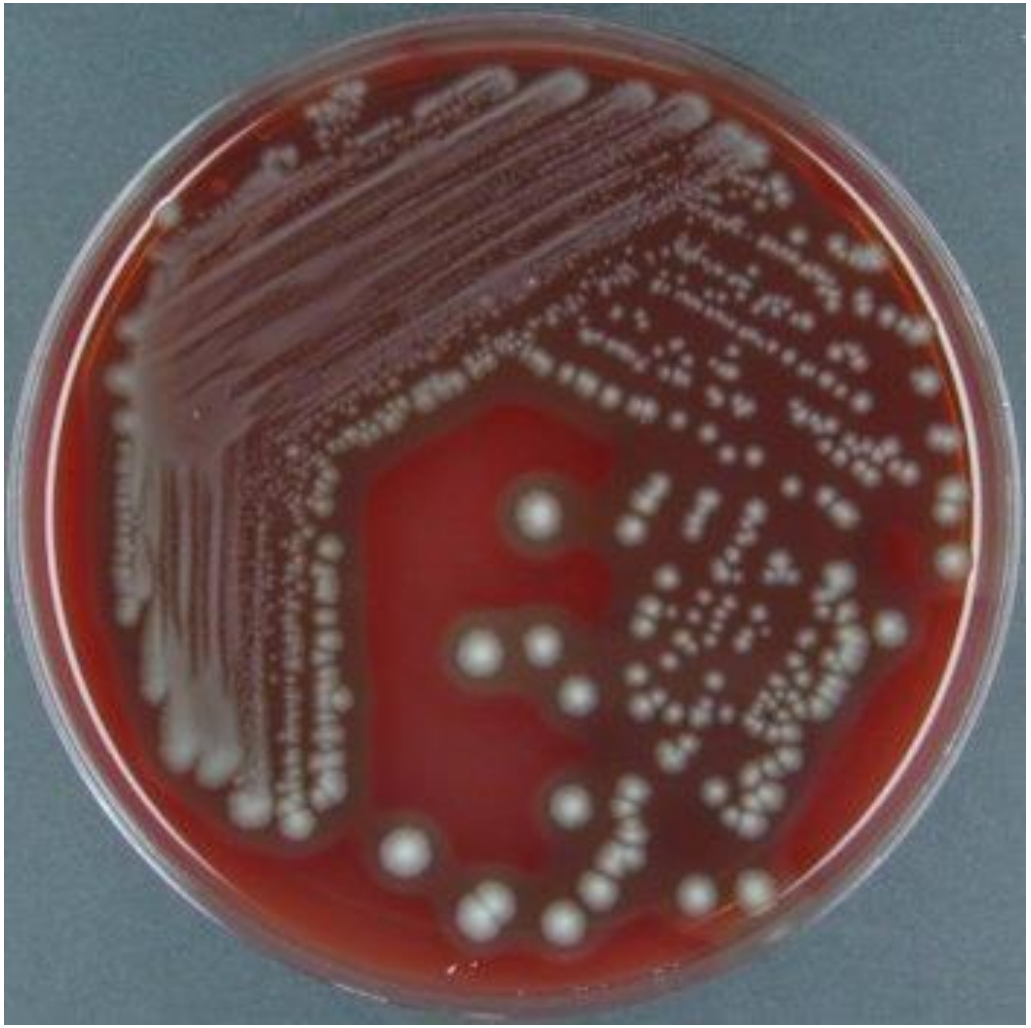


P.acnes (pure culture smear, Gram stain)

Fusobacterium necrophorum



колонии ***Fusobacterium necrophorum***
on blood agar



Legionellae

- A widely publicized outbreak of pneumonia in persons attending an American Legion convention in Philadelphia in 1976 prompted investigations that defined *Legionella pneu- mophila* and the legionellae. Other outbreaks of respiratory illness caused by related organisms since 1947 have been diagnosed retrospectively. Several dozen species of *Legionella* exist, some with multiple serogroups. *L pneumophila* is the major cause of disease in humans; *Legionella micdadei* and a few other species sometimes cause pneumonia. The other legionellae are rarely isolated from patients or have been isolated only from the environment.

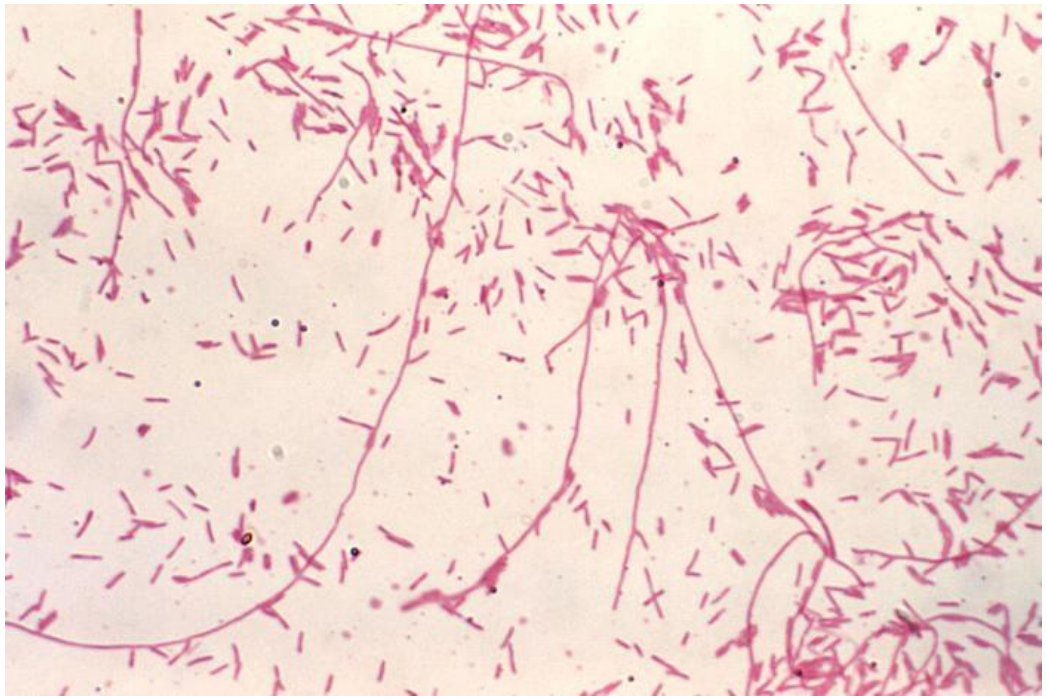
Legionella

- *Class - Gammaproteobacteria*
- *Family - Legionellaceae*
- *Genus - Legionella*
- *Species – – 9 species of which the main human pathogen is: Legionella pneumophila*

-
- *Opening history:*
- *1977 - D. McDade and S. Shepard, CDC. Atlanta (USA)*

Legionella pneumophila

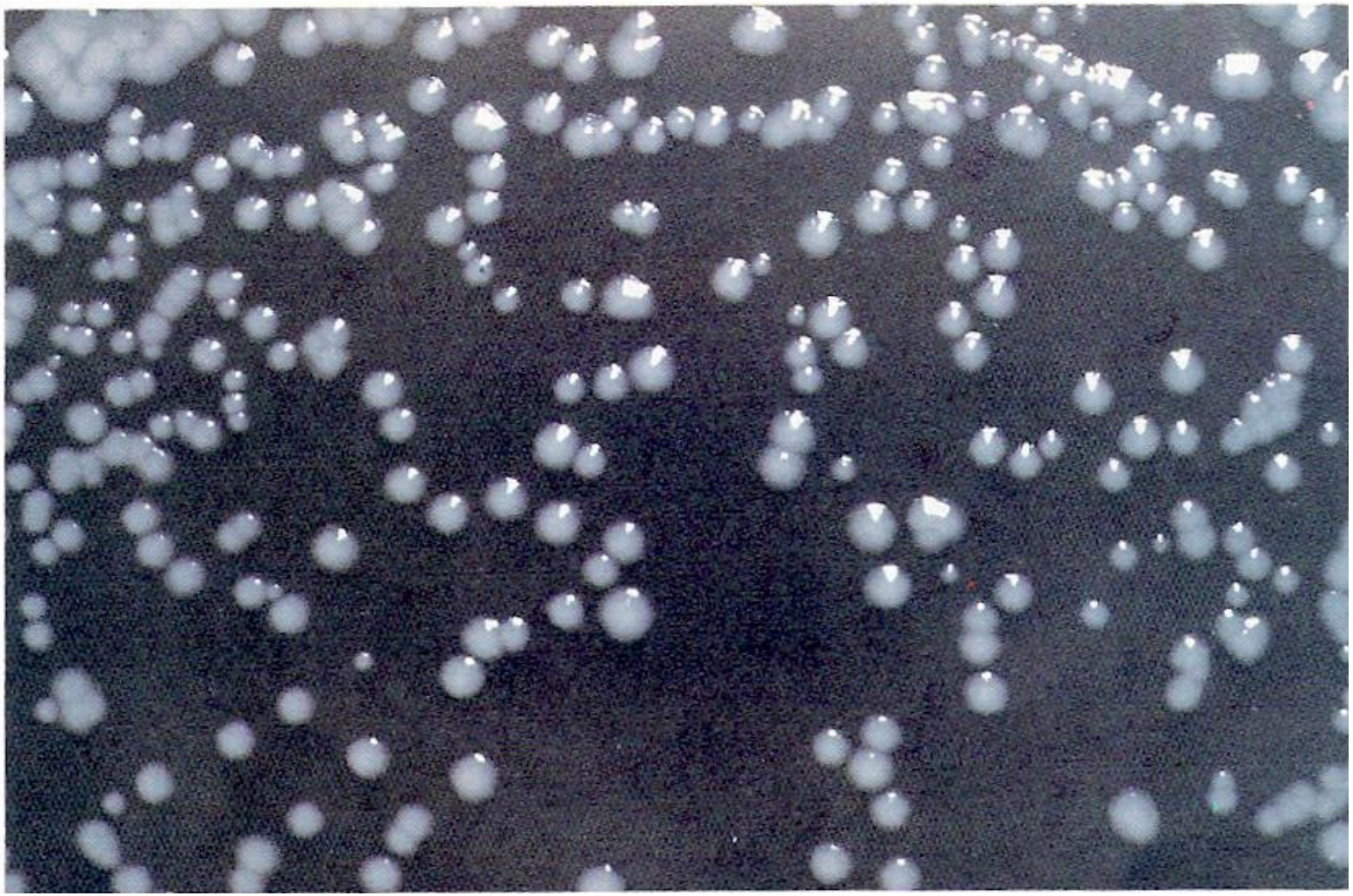
Gram-negative rods, 2-3x0.5-0.7 μm in size, may have a filamentous form. They have flagella and pili; they do not form spores or capsules. Contains fatty inclusions in the form of unique fatty acids



Culture and Growth Characteristics

- Legionellae can be grown on complex media such as buffered charcoal yeast extract agar with α-ketoglutarate, L-cysteine, and iron (BCYE) at a pH of 6.9, temperature of 35°C, and 90% humidity. Antibiotics can be added to make the medium selec- tive for *Legionella* species. The charcoal acts as a detoxifying agent. Legionellae grow slowly; visible colonies are usually present after 3 days of incubation. Colonies that appear after overnight incubation are not *Legionella* species. Colonies are round or flat with entire edges. They vary in color from color- less to iridescent pink or blue and are translucent or speckled.

Legionella pneumophila
culture on charcoal-yeast agar



Biological properties of legionella

Reaction	*L. worsleiensis*	*L. quateirensis*	*L. geestiana*	*L. londiniensis*	*L. nautarum*
Hippurate hydrolysis	−[a]	−	+[w]	− or +[w]	−
D-Glucose (acid production)	−	−	−	−	−
Catalase activity	−	+	+	+[w]	+
Oxidase activity	−	−	−	−	+
Peroxidase activity	+	+	+[w]	−	+
Urease activity	−	−	−	−	−
Browning with the following substrates:					
Tyrosine	+	+	+[w]	+	−
3,4-Diaminobenzoic acid	+[w]	+	−	−	−
3,5-Diaminobenzoic acid	+	+	−	−	−
Autofluorescence	−	−	−	−	−
Bromocresol purple spot test	−	−	−	−	−
β-Lactamase activity	+	+	−	+	+
Elastase production	−	−	+	+	−
Casein hydrolysis	+	+	+	+[w]	−
Albumin degradation	+	+	+	+[w]	−
Chondroitin sulfate degradation	+	+	+	−	−
Starch hydrolysis	−	+[w]	−	−	−
DNase activity	+	+	+	+	−
Gelatin hydrolysis	+	+	+	+	−
Lipase activity	−	+	−	−	+
Protease activity	−	+	−	−	−
Alkaline phosphatase activity at:					
pH 9.5	+	+	+	+	+
pH 10.5	+	+	+	+	+
Single polar flagellum	+	+	+	−	−
Requirement for cysteine	+	+	+	+	+
Growth on:					
Legionella blood agar	−	−	−	−	−
Mueller-Hinton agar	−	−	−	−	−
BCYE agar	+	+	+	+	+
Nitrate reduction	−	−	−	−	−

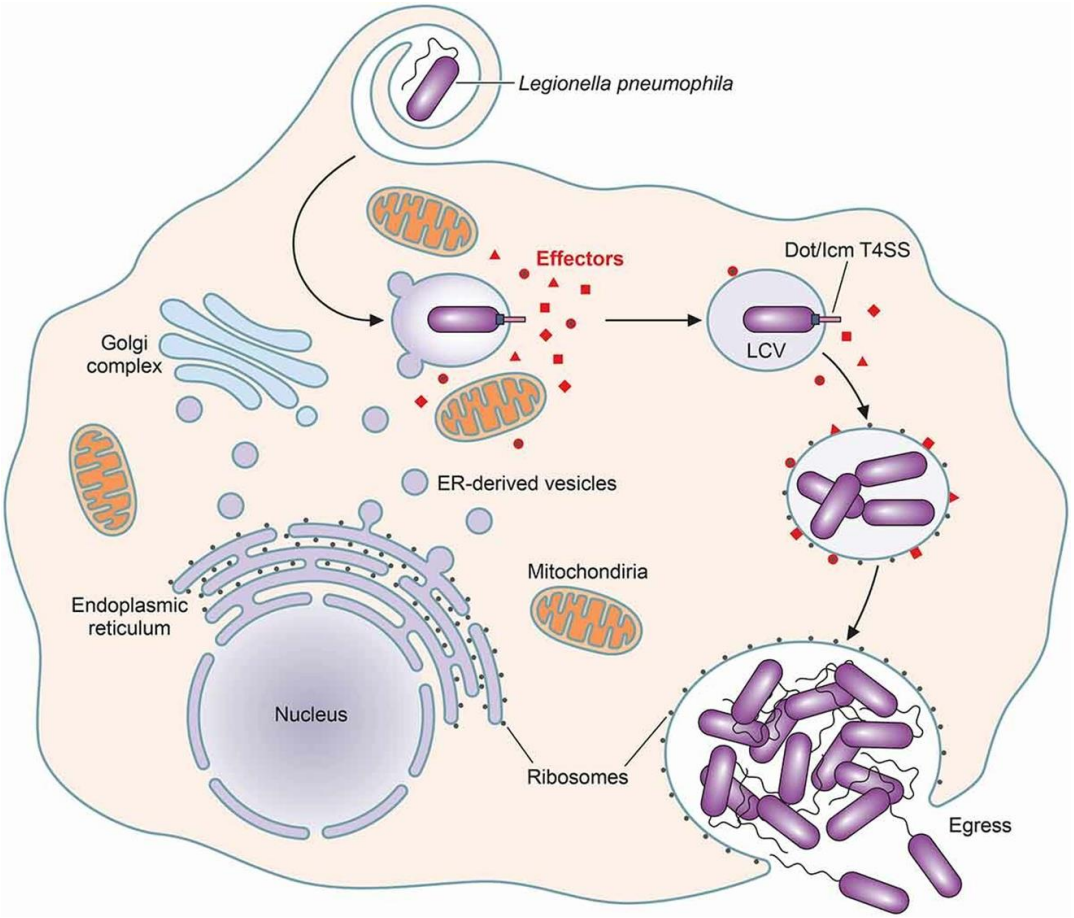
[a] +, positive; −, negative; +[w], weakly positive.

- They have group-specific and type-specific antigens. There are 8 serogroups.

Legionella pathogenicity factors

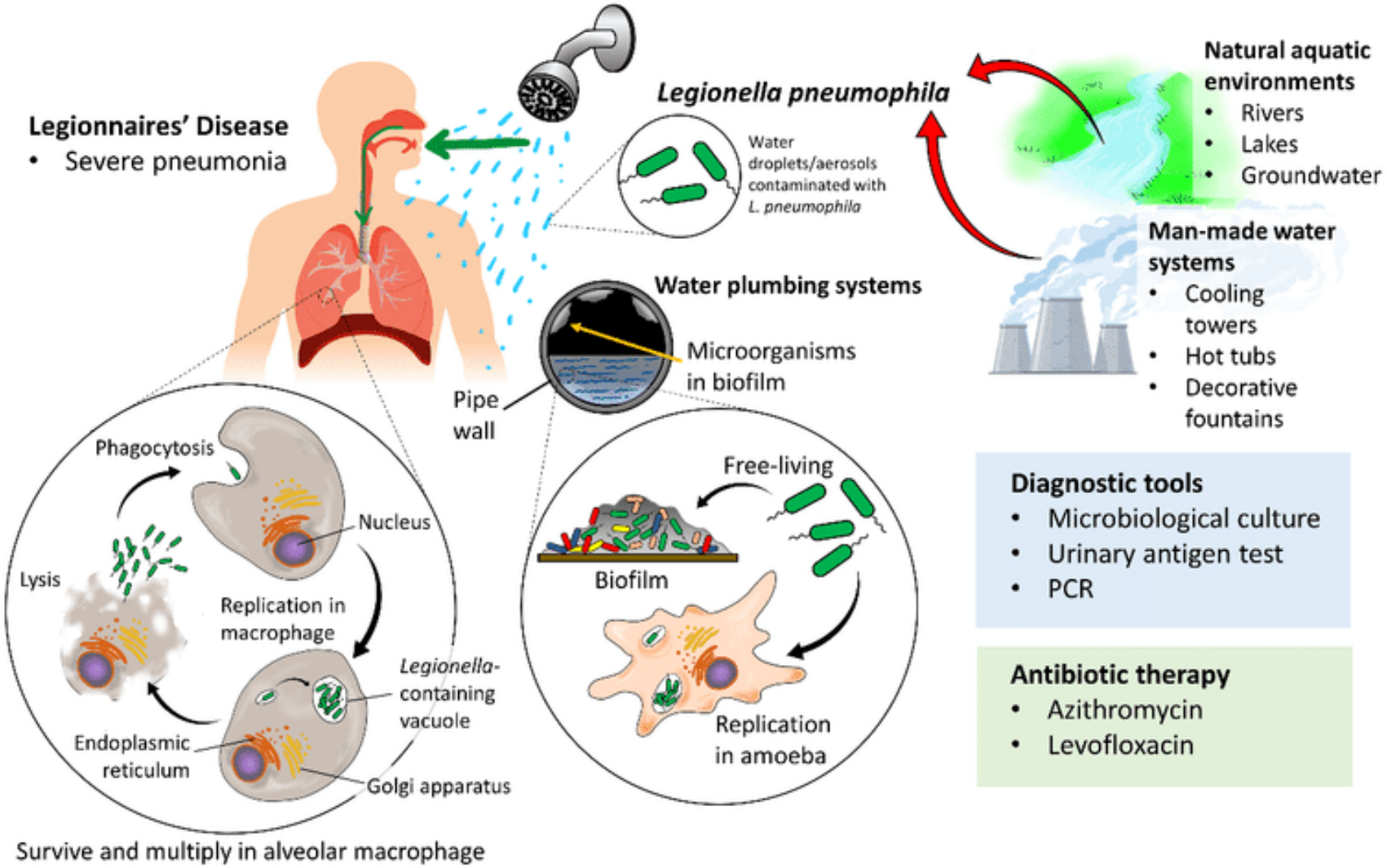
- Legionella are facultative intracellular parasites. In the human body, they multiply mainly in alveolar macrophages, which they enter as a result of inhalation of microbial aerosol, as well as in polymorphonuclear leukocytes and blood monocytes.
- Active reproduction in macrophages is provided by the following pathogenicity factors:
- cytotoxin and superoxide dismutase, which suppress the "respiratory burst" of the phagocyte;
- cytolysin, which is a metalloprotease enzyme that prevents the formation of phagolysosomes, and also causes a hemorrhagic effect.
- When bacteria die, endotoxin is released, causing intoxication.

Legionella life cycle



EPİDEMİOLOGY

- Peak season for *Legionella* infections is late summer to autumn.
- Travel, particularly on cruise ships, may be a risk factor.
- Transmission is usually the result of
  - inhalation or
  - ingestion followed by aspiration of aerosols from contami- nated water systems.
- The natural habitats for legionellae are
  - Lakes
  - Streams
  - Rivers
  - especially thermally heated bod- ies of water
  - soil.


Legionnaires' Disease
• Severe pneumonia
Legionella pneumophila
Water droplets/aerosols contaminated with L. pneumophila
Natural aquatic environments
• Rivers
• Lakes
• Groundwater
Man-made water systems
• Cooling towers
• Hot tubs
• Decorative fountains
Water plumbing systems
Microorganisms in biofilm
Pipe wall
Phagocytosis
Nucleus
Lysis
Replication in macrophage
Legionella-containing vacuole
Endoplasmic reticulum
Golgi apparatus
Free-living
Biofilm
Replication in amoeba
Survive and multiply in alveolar macrophage
Diagnostic tools
• Microbiological culture
• Urinary antigen test
• PCR
Antibiotic therapy
• Azithromycin
• Levofloxacin

Microbiological diagnosis of legionella infection

- *Microscopic method - microscopy of Gram-stained smears made from pathological material.*
- *The bacteriological method is the isolation of a pure culture of the pathogen on the Muller-Hinton medium.*
- *Biological test - infection of guinea pigs and mice.*
- *Serological reactions - an indirect immunofluorescence reaction is used to determine antibodies. RPGA, ELISA, RSK,*
- *Accelerated diagnosis - tests to determine the antigen in the blood and urine using ELISA, RIA.*
- *Molecular genetic method - determination of microbe DNA using PCR.*

hydrosense®

Reliable self testing
for *Legionella*

Comparison of *Legionella* testing techniques: Hydrosense®, PCR and Culture

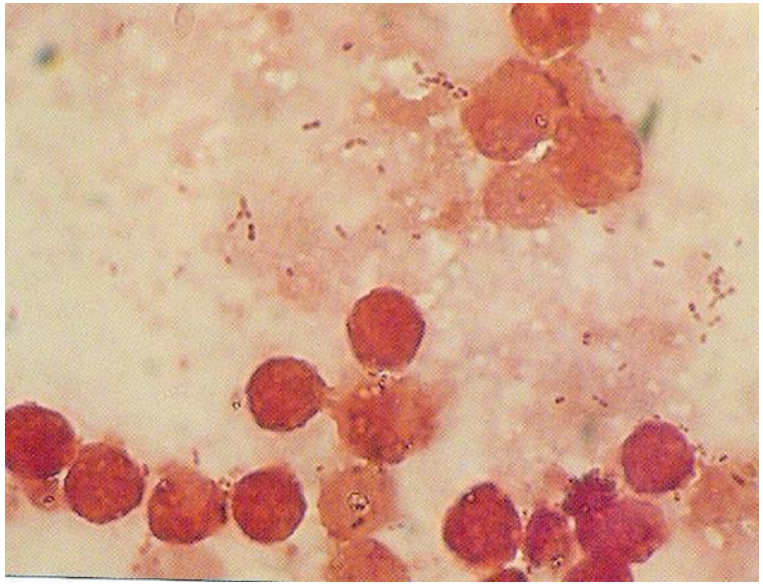
	HYDROSENSE®	PCR	CULTURE
Sensitivity to dangerous strains of *Legionella*	High	High	Moderate to High
What is being measured?	*Legionella pneumophila* Antigen	*Legionella pneumophila* DNA	*Legionella pneumophila* Colony Forming Units – CFU
Reliability (Recovery Rate)	80% – 90%	80% – 90%	10% – 60%
Detects dangerous form of *Legionella pneumophila*	Yes	Yes	No
Level of expertise required	Low	High	High
On-site result	Yes	No	No
Speed of result	25 minutes	1 – 3 days (depending on transport)	10 – 14 days
Results certificate immediately available	Yes	No	No

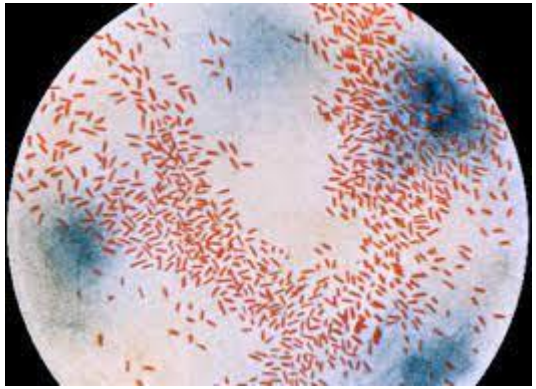
Hemophilus bacteria

- *Class - Gammaproteobacteria*
- *Family - Pasteurellaceae*
- *Genus - Haemophilus*
- *Species - 8 species of which the main human pathogens are: Haemophilus influenzae*
- *Haemophilus ducreyi*
- *The history of the discovery of Haemophilus influenzae:*
- *1891 - M.I. Afanasiev isolated the pathogen from a patient who died of influenza*
- *1892 - R. Pfeiffer and S. Kitazato described the pathogen*
- *Discovery of Haemophilus ducreyi:*
- *1887 - O.V. Peterson discovered the pathogen*
- *1890 - A. Ducret described the microbe in detail*

Morphology and tinctorial properties of H. influenzae

- Small, gram-negative, spherical, ovoid, or rod-shaped bacteria, sometimes forming pairs, short chains, or filaments.
- They are motionless, do not form spores, have drank. The formation of a capsule is a variable sign, and its detection can serve as a kind of marker of the virulence of the strain..

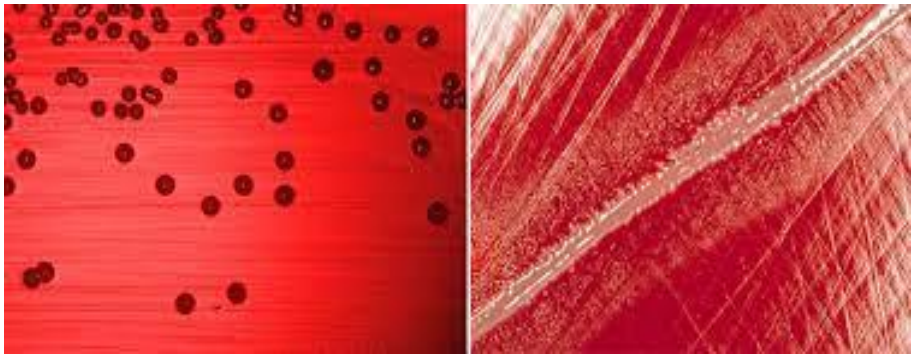




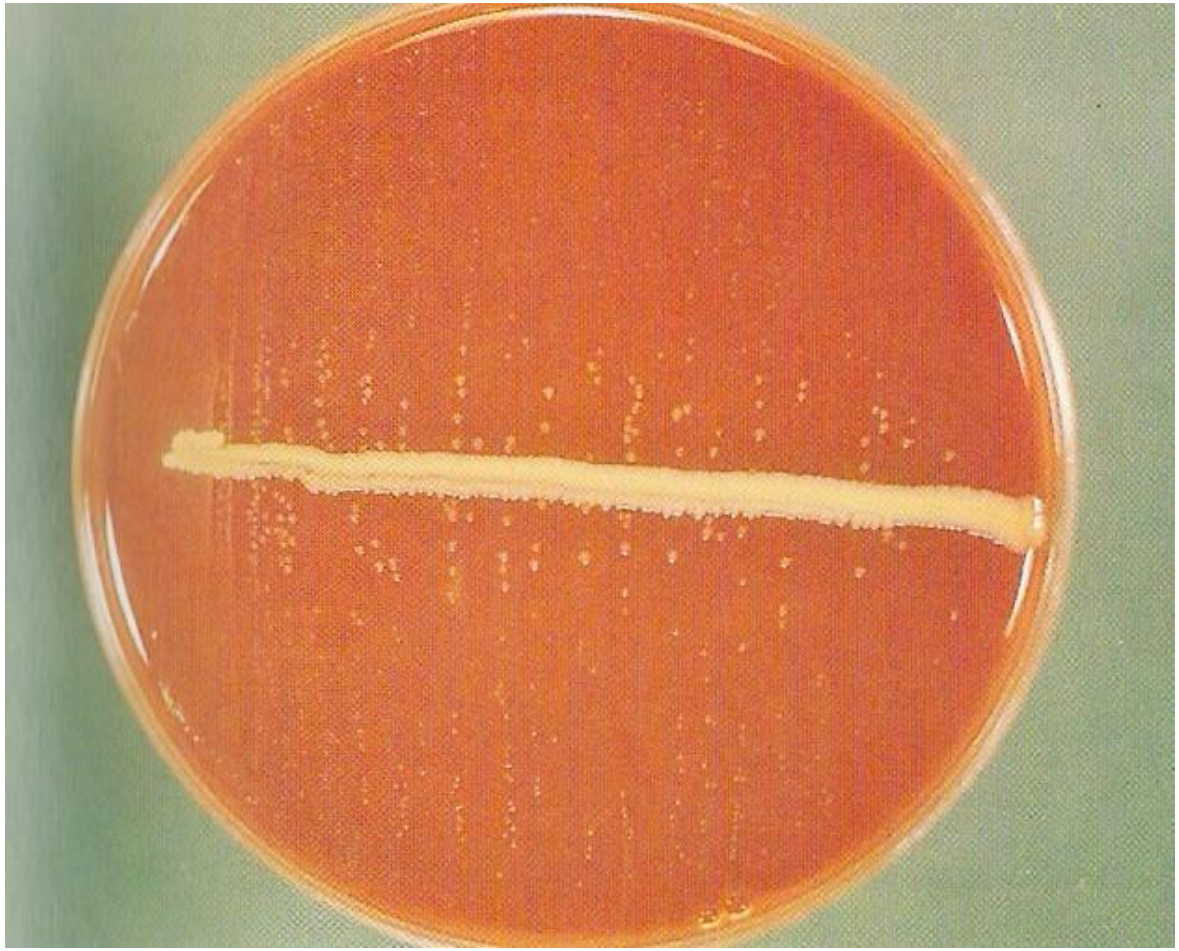
cultural properties

- On blood agar, hemophilus bacteria do not grow or grow poorly. Therefore, for the cultivation of Haemophilus influenzae, chocolate agar is used, obtained by heating blood agar at 80 °C, resulting in hemolysis and release of hemin and NAD from erythrocytes and inactivation of NADase enzymes.
- Hemophilus bacteria are characterized by the so-called feeder phenomenon, or satellite phenomenon, which is manifested in their ability to grow on blood agar around colonies of staphylococci or other bacteria that produce NAD or cause hemolysis. For the hemophilic rods themselves, the ability to cause hemolysis is uncharacteristic

Haemophilus influenzae



"Feeder Phenomenon" The ability of Haemophilus influenzae to grow on blood agar around Staphylococcus colonies



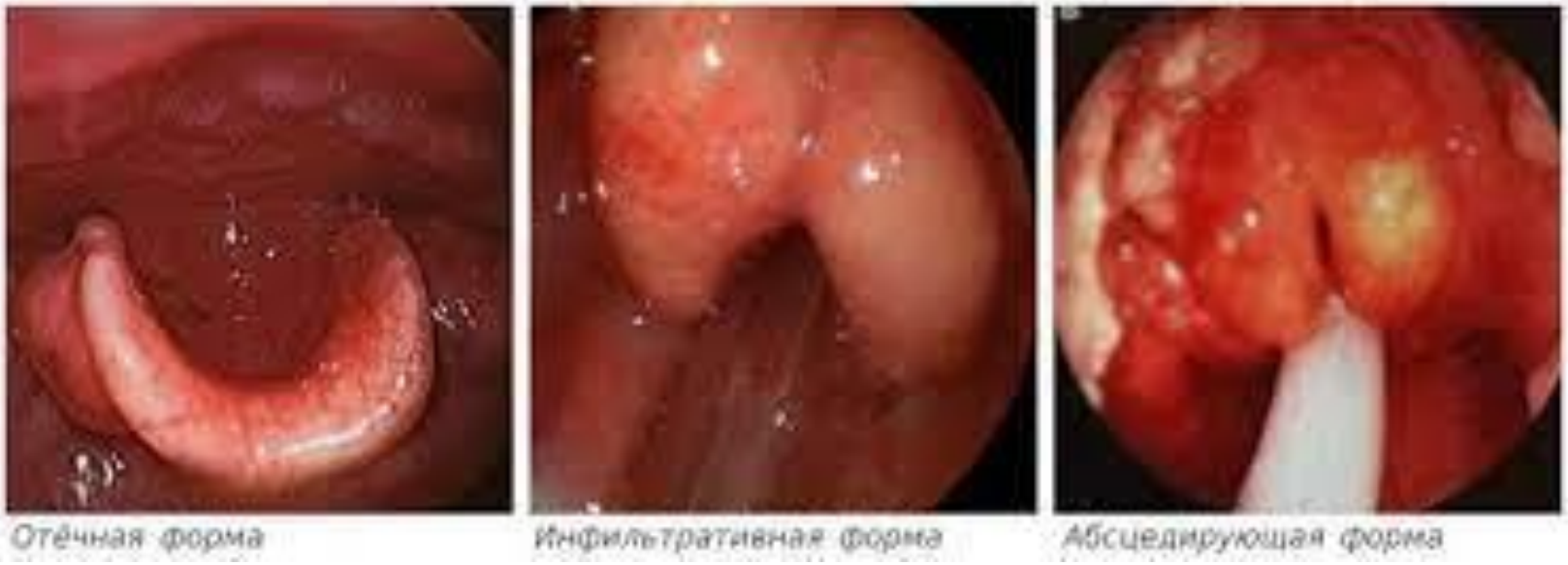
Biological properties of Haemophilus influenzae

- **biochemical activity. Hemophilic bacteria are chemoorganotrophs. Metabolism is respiratory and fermentative. Utilize glucose to acid, reduce nitrates to nitrites. Other carbohydrates ferment poorly**.
- **antigenic properties. H. influenzae have an O-antigen and a capsular polysaccharide K-antigen, depending on the structure of which six serotypes are distinguished - a, b, c, d, e, f. The chemical composition of the capsular antigen of Haemophilus influenzae serotype b is unique: it is a polymer of ribose and ribitol - polyribozoribitol phosphate (PRP).** Capsular haemophilus variants can be typed using the "capsule swelling" test or in RIF with specific sera. Most variants of H. influenzae, which are part of the normal microflora of the upper respiratory tract, are non-capsular forms, which are commonly called "non-typable".

Pathogenicity factors of Haemophilus influenzae

- **Adhesion pili that ensure the penetration of the microbe into macrophages**
- **IgA protease - inactivates secretory antibodies**
- **Capsule - protects against phagocytosis by suppressing the oxidative burst.**
- **Endotoxin - participates in the processes of adhesion and invasion, causes paralysis of the epithelium of the respiratory tract.**

According to world statistics, hemophilic infection (Hib-infection) occupies one of the first places among the causes of child mortality. Mortality in purulent meningitis, sepsis and epiglottitis in the absence of adequate treatment is 90%.



Отёчная форма | Инфильтративная форма | Абсцедирующая форма

Epidemiology and pathogenesis of infection caused by Haemophilus influenzae

- Source of infection
- - a sick person or a bacteriocarrier.
- Transfer mechanism
- - respiratory
- Transmission route
- - airborne.
- Pathogenesis disease is associated with the presence or absence of the capsule.
- Non-capsular variants lead to asymptomatic carriage or local HL - otitis media, sinusitis, laryngotracheitis, bronchitis, pneumonia.
- Capsular variants (type b) cause septicemia, arthritis, endocarditis, epiglottitis, laryngotracheitis, purulent meningitis.

Microbiological diagnosis of infection caused by Haemophilus influenzae

- ***Microscopic method***
- – microscopy of Gram-stained and Romanovsky-Giemsa smears made from pathological material.
- ***Bacteriological method –*** isolation of a pure culture of the pathogen with identification and differentiation.
- ***Serological reactions***– agglutination and precipitation reactions.
- **Accelerated Diagnostics-** tests for the determination of antigen by methods of counter immunoelectrophoresis, RIF, latex agglutination reaction, “capsule swelling” test.

Specific prevention of infection caused by Haemophilus influenzae

- To create artificially acquired active immunity against H. influenzae type b, a subparticulate vaccine containing a purified capsular antigen (PRP) has been developed. However, the Hib polysaccharide vaccine has low immunogenicity because it contains a T-independent antigen. Therefore, vaccination of children is carried out starting from 1.5 years of age.
  To increase the effectiveness of vaccination against Hib infection, it has been proposed to use conjugate vaccines containing capsular antigen (PRP) on a carrier protein. As such carriers, diphtheria, tetanus toxoids or proteins of the outer membrane of group B meningococcus (combined vaccine for the prevention of meningococcal and hemophilic meningitis) are used.
- Passive immunization with donor serum preparations containing high concentrations of IgM may be given to children with a weak immune response to the vaccine and immunodeficient individuals.

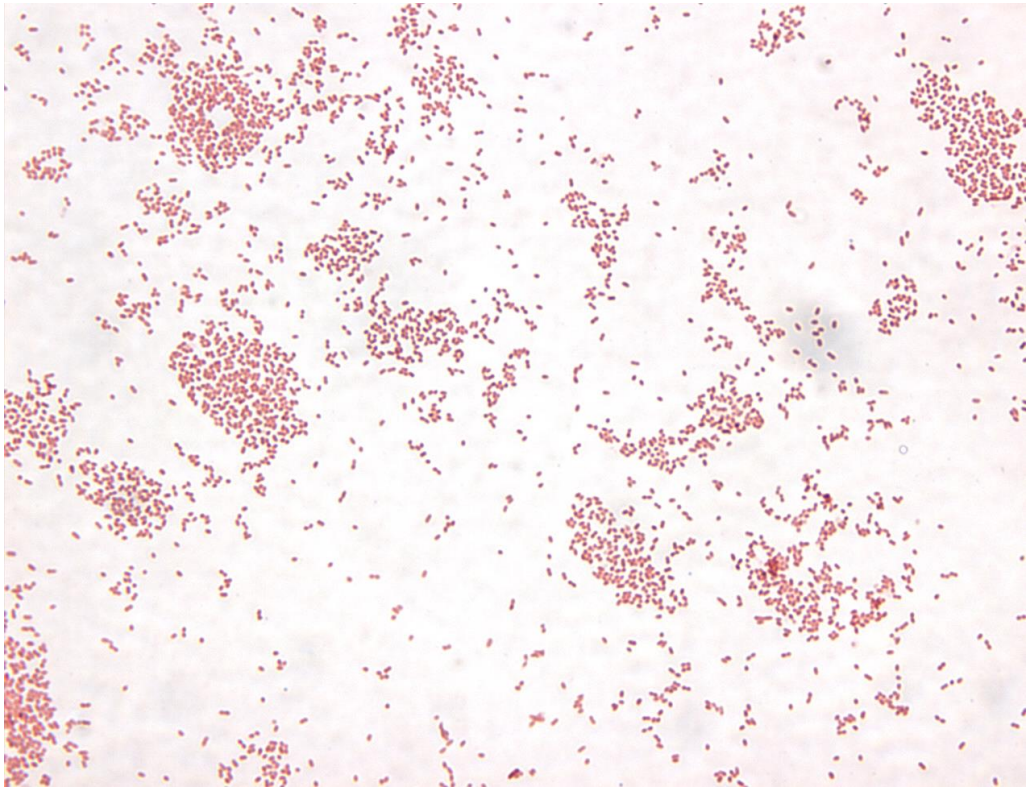
Bordetella (Genus Bordetella)

- For humans, pathogenic B.pertussis and B.parapertussis, which cause whooping cough and parapertussis, respectively.
- *B. bronchiseptica cause diseases in animals, in particular in dogs (pertussis-like disease). Occasionally, respiratory infections and bacteremia are caused in an immunocompromised person.B.avium* cause disease in birds, particularly turkeys. Other species of the genus: B.binzii cause respiratory infections and bacteremia, B.bolmseii - bacteremia in immunocompromised individuals, B.trematum - otitis media and wound infections.

Morpho-biological properties

B.pertussis və
B.parapertussis –

very small Gram-negative coccobacilli that form a capsule. They are motionless, do not form a dispute.



B.pertussis (in pure culture swab)

cultural properties

- obligate aerobes
- For cultivation, casein-coal agar (CUA) or Borde-Gangu medium (potato-glycerol agar with the addition of 20% blood and 0.5 μg / ml of penicillin G) are used.
- Crops are incubated at a temperature of 35-370C for 3-7 days at high humidity.
- On Borde-Jangu medium, B.pertussis forms small grayish colonies resembling drops of mercury or pearls, in virulent strains - with a small area of hemolysis.
- B.parapertussis, B.bronchiseptica, B.avium and other species can grow on simple nutrient media, forming visible colonies after 1-2 days of cultivation.

Bordetella pertussis

(small grayish shiny colonies resembling drops of mercury or pearls on casein charcoal agar)

Colonies on casein charcoal agar (CAA)

Antigenic structure:

- ***Generic antigens***– being a thermostable somatic O-antigen, they mediate the agglutination of bacteria by homologous and heterologous antisera.
- ***Species antigens***– is a thermolabile K-antigen, consisting of 14 fractions designated by Arabic letters. The 7th factor is common to all bordetells. Specific for B.pertussis is the 1st factor, for B.parapertussis - the 14th factor, and for B.bronchiseptica - the 12th.
- The K-antigen of bordetell is detected by an agglutination test, called agglutinogen.

Intraspecific differentiation of the genus Bordetella

signs	B.pertussis	B.parapertussis	B.bronchiseptica
Mobility	-	-	+
Growth on simple nutrient media	-	+	+
Pigment formation	-	+	-
Duration of cultivation on Borde-Jangu medium	3-6 days	2-3 days	1-2 days
Urease oxidase catalase Nitrate recovery	- + + -	+ - + -	+ + + +
Specific Thermolabile antigen 1st factor 12th factor 14th factor	 + - -	 - - +	 - + -

Pathogenic factors:

- *Virulent genes of B.pertussis: bvgA və bvgS*
- ***adhesion factors and*** toxins play a role in the adhesion of bacteria to the ciliated epithelium of the respiratory tract (bronchi, trachea)
- ***Microvilli (pili, fimbriae)***

-cover the surface of cells of virulent strains of B.pertussis, are agglutinogens.

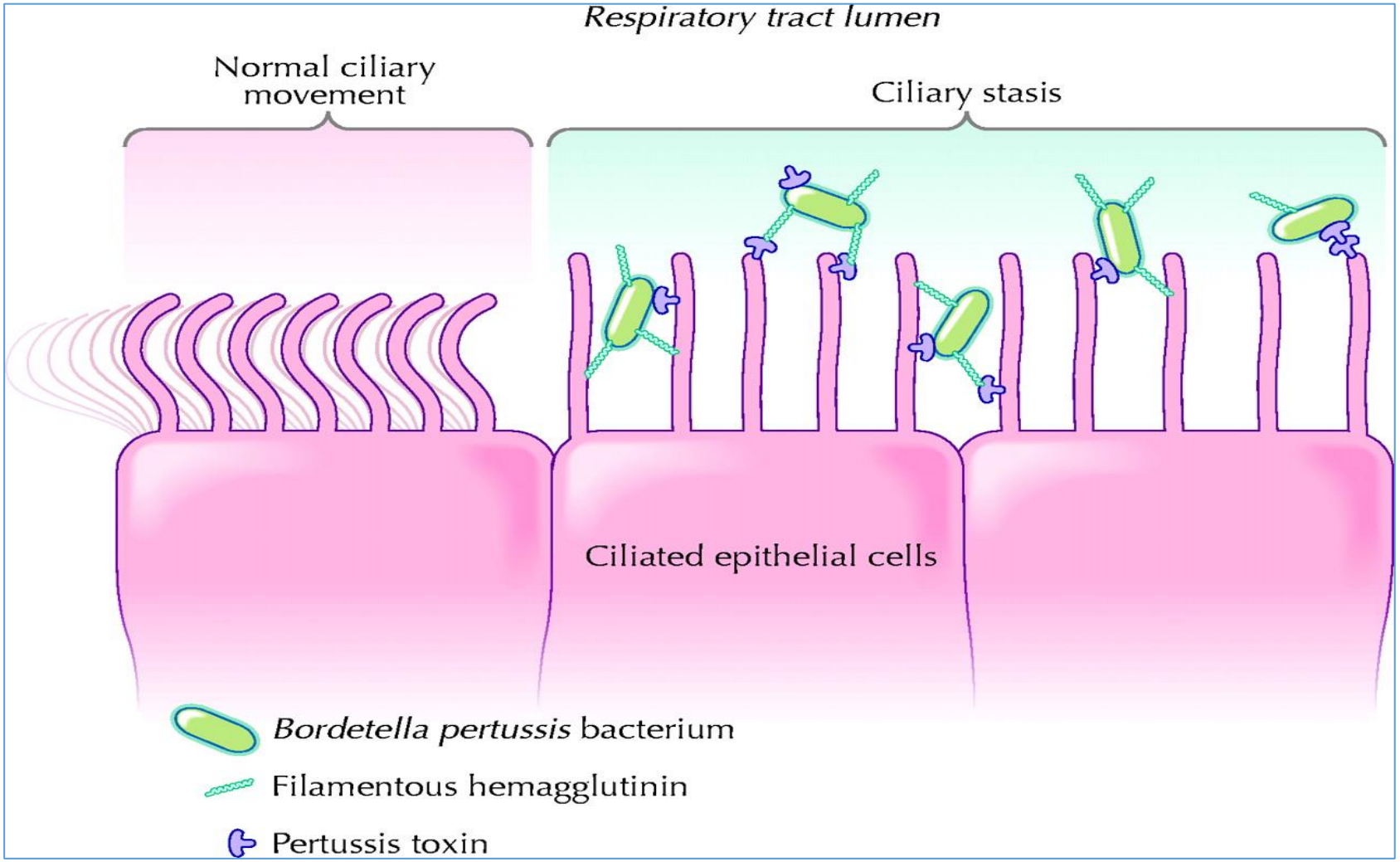
Filamentous hemagglutinin- a protein capable of selectively binding to glycolipid receptors of the cilia of the epithelium of the trachea and bronchi.

Pathogenic factors:

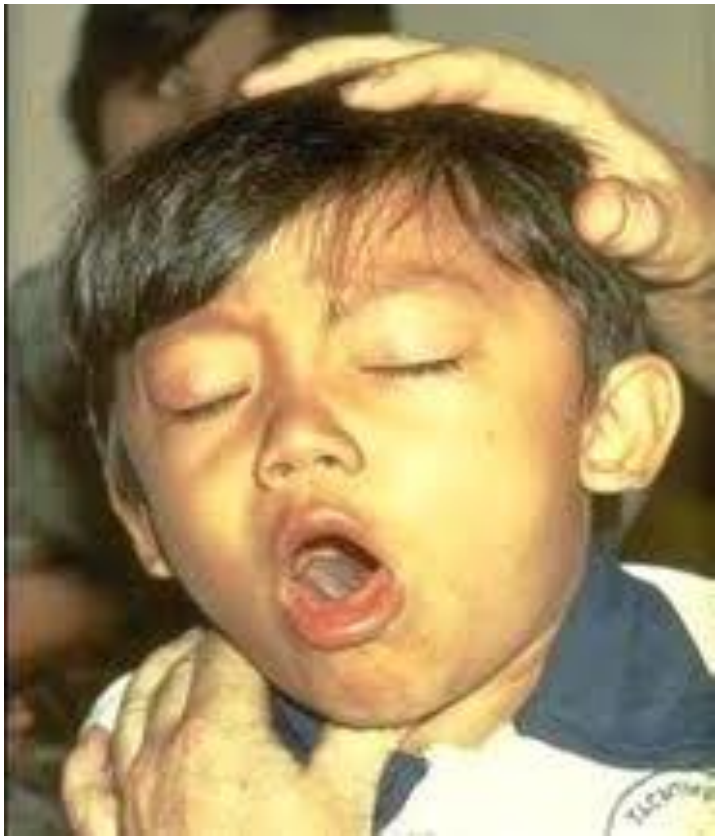
- ***Pertussis toxin****(pertussin, exotoxin)* **-** *stimulates the work of adenylate cyclase in the cells of the respiratory tract by inhibiting the regulatory protein G. The accumulation of cAMP in cells leads to disruption of their function.*
- ***Specific (tracheal) cytotoxin–*** inhibits DNA synthesis in epithelial cells of the respiratory tract, leading to cell death and desquamation.
- ***Endotoxin*** **–** stimulates the production of cytokines, causing a damaging effect on the epithelium of the respiratory tract.

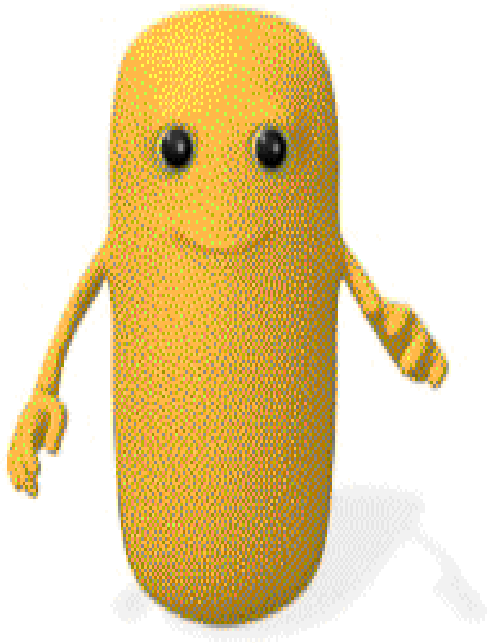
Bordetella pertussis

pathogenicity factors and pathogenesis



With whooping cough, attacks of spastic cough are accompanied by severe hypoxia, cyanosis, convulsive syndrome, and often ends in vomiting.





Microbiological diagnostics

- Research material - mucus from the posterior pharyngeal wall
- ***Bacteriological method***– material from the nasopharynx is taken using special calcium alginate swabs moistened with a solution of penicillin, or using the “cough plates” method - during a coughing fit, a Petri dish with Borde-Gangu medium is held directly in front of the patient's face.
- Serological method - RIF is used with specific fluorescent serum and material from the patient's nasopharynx.
- ***Molecular genetic method (PCR)***– the test is carried out using primers B.pertussis and B.parapertussis.

Specific prophylaxis

- ***DTP vaccine*** (adsorbed pertussis-diphtheria-tetanus toxoid). Immunization is carried out starting from the age of 3 months, three times, with intervals between the administration of the drug at 4-6 weeks.
- For emergency prevention of whooping cough in persons in contact with patients, normal human immunoglobulin and erythromycin are prescribed in the first 5 days after contact with the diseased.

Treatment

- *Erythromycin - used in the catarrhal stage.*
- *Normal human immunoglobulin - used to treat severe or complicated forms of whooping cough.*
- *oxygen inhalation.*
- *Antihistamines or sedatives.*