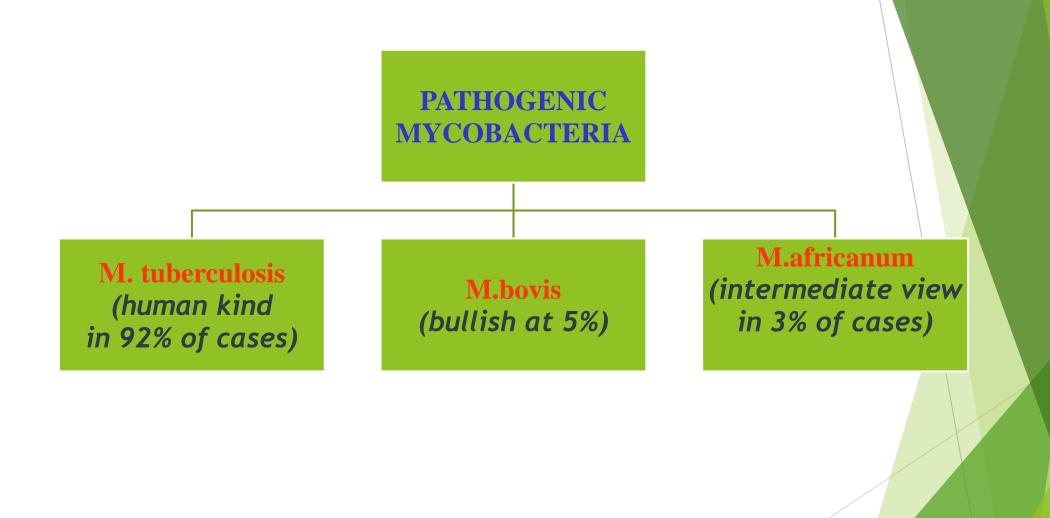
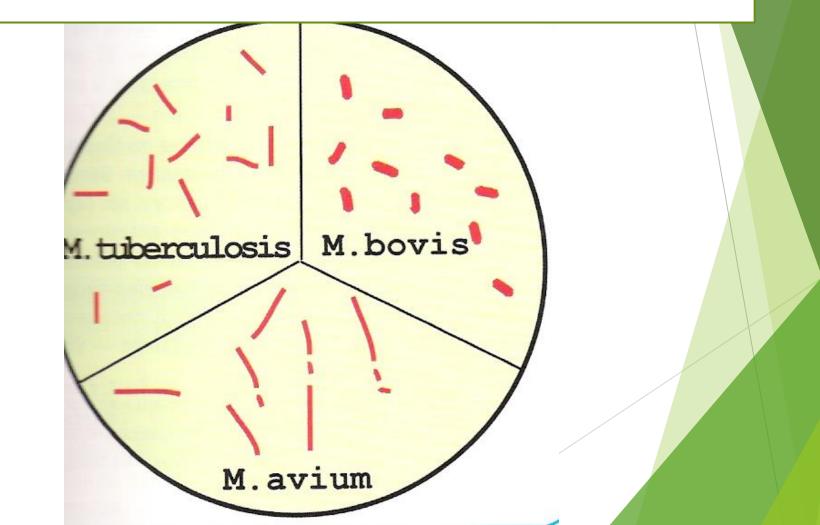
# Lesson 17

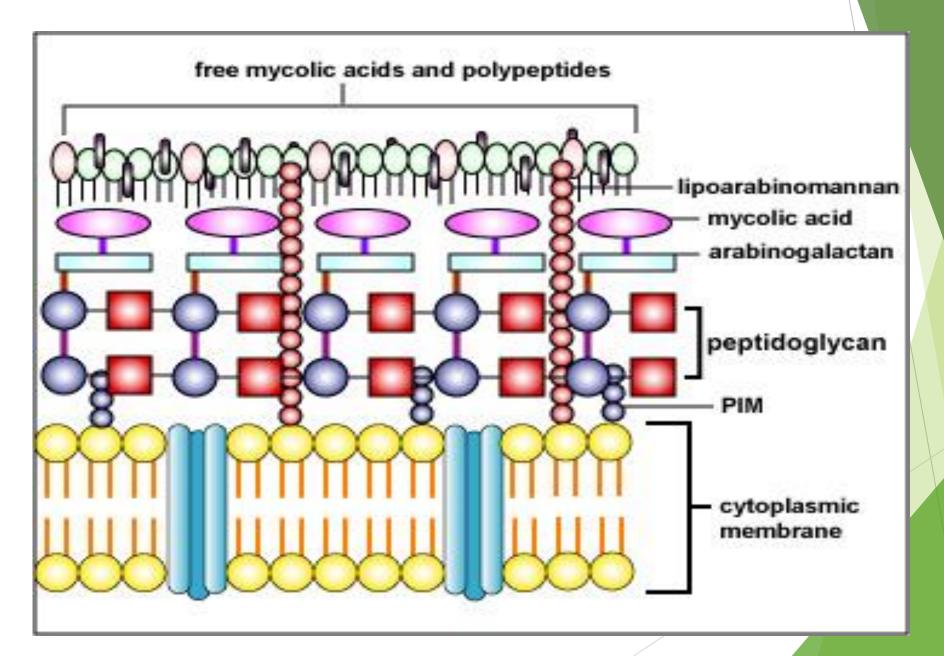
Microbiological diagnosis of diseases caused by bacteria of the genera Mycobacterium, Actinomyces, Nocardia and Mycoplasma.



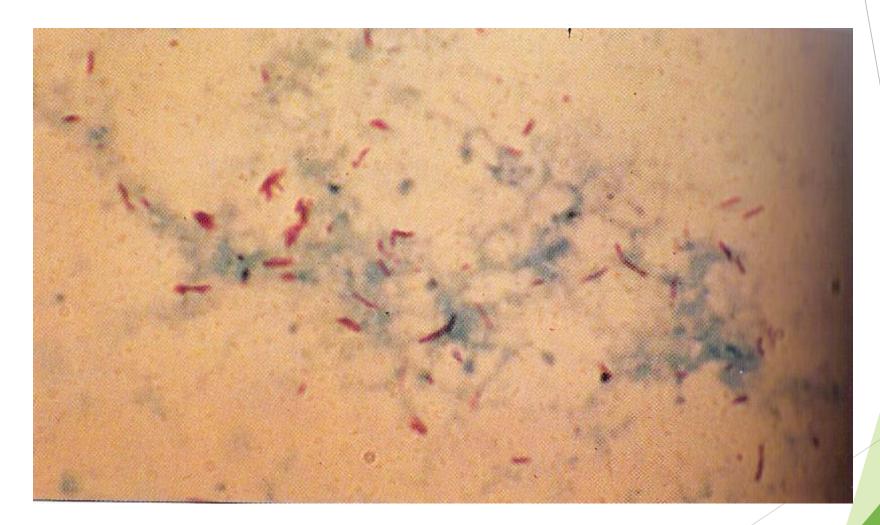
Acid-, alcohol-, alkali-resistant polymorphic immobile rods with homogeneous or granular cytoplasm, gram (+), do not form spores, have a microcapsule



#### The structure of the cell wall of acid-fast bacteria



#### Mycobacterium tuberculosis in sputum preparation (Ziehl-Neelsen stain)

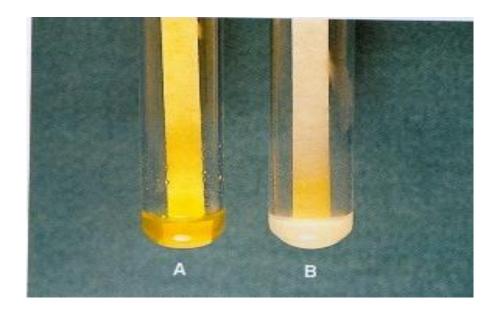


#### **Biochemical properties of pathogenic mycobacteria**

Activity	M.marinum	M.tuberculosis	<b>M.bovis</b>	M.avium	M.kansasii	M.scroful aceum
Urease	+	+	+	_	+	+
Pyrazinamidase	+	+	_	+	±	+
Acid phosphatase	+	+	+	_	+	+
Nitrate recovery	_	+	_	_	+	±
α- esterase	_	+	+	+	_	±
Catalase	±	—	—	±	+	+
Synthesis niacin	—	+	_	—	_	

#### Mycobacterium tuberculosis (biochemical features)

Niacin test - Niacin test - Unlike M. bovis and opportunistic mycobacteria, M. tuberculosis produces nicotinic acid (niacin). The test is based on the formation of a complex compound of potassium cyanide and a solution of chloramine B with nicotinic acid, which gives a bright yellow color.



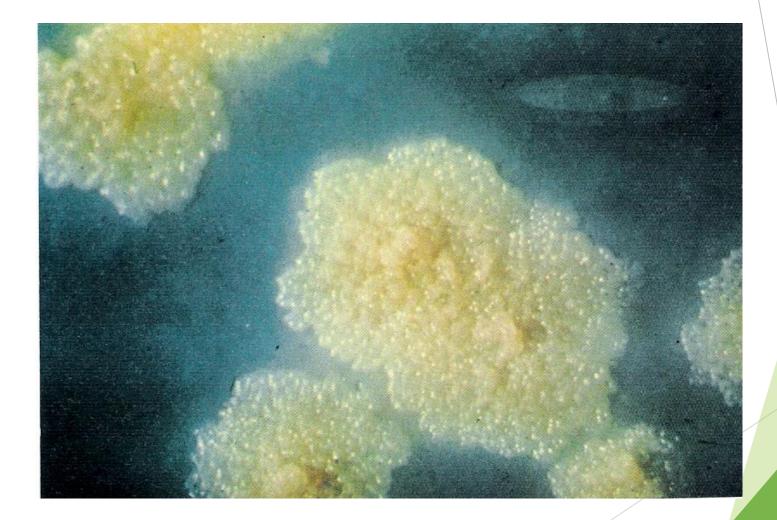
Niacin test: A-positive; B-negative

When growing on liquid nutrient media, virulent strains of Mycobacterium tuberculosis have a cord factor



- Weakens the migration of leukocytes- Participates in the development of chronic granuloma- Acts as an immunological adjuvant

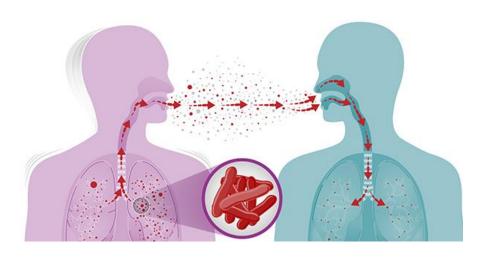
#### **Mycobacterium tuberculosis (culture on Lowenstein-Jensen medium)**



# Pathogenicity factors of Mycobacterium tuberculosis

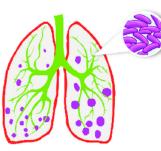
Tuberculoproteins - cause the development of hypersensitivity reactionsLipid complex--- mycolic acid-- phthionic acid causes the development of granulomas and caseous necrosis, suppression of phagocytosis-- cord factor - prevents the formation of phagolysosomes

# Transmission





Step 5: Infection, with potential to progress to disease



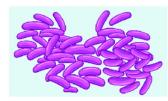
Step 1: Source case Individual with active tuberculosis



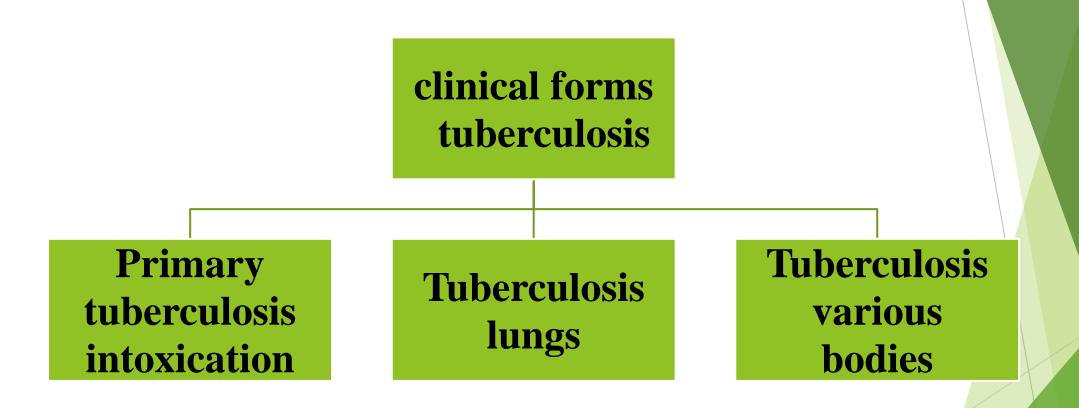
Step 2: Aerosolization Release of viable airborne bacilli



Step 4: Exposure and inhalation Contact: nature, duration, intensity, locations



Step 3: Airborne survival Characteristics and factors associated with survival of Mycobacterium tuberculosis in air



# **Tuberculosis Symptoms**



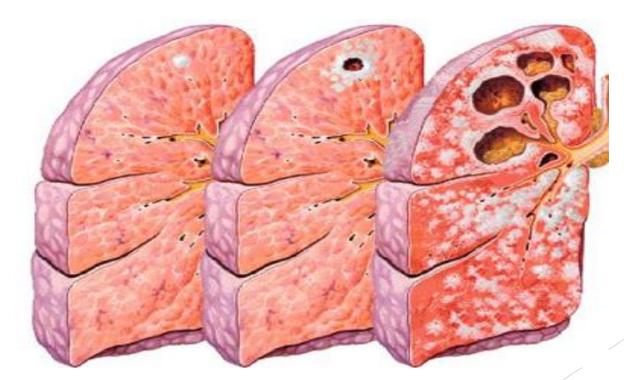
# **Development of Tuberculosis (TB)**

Mycobacterium tuberculosis

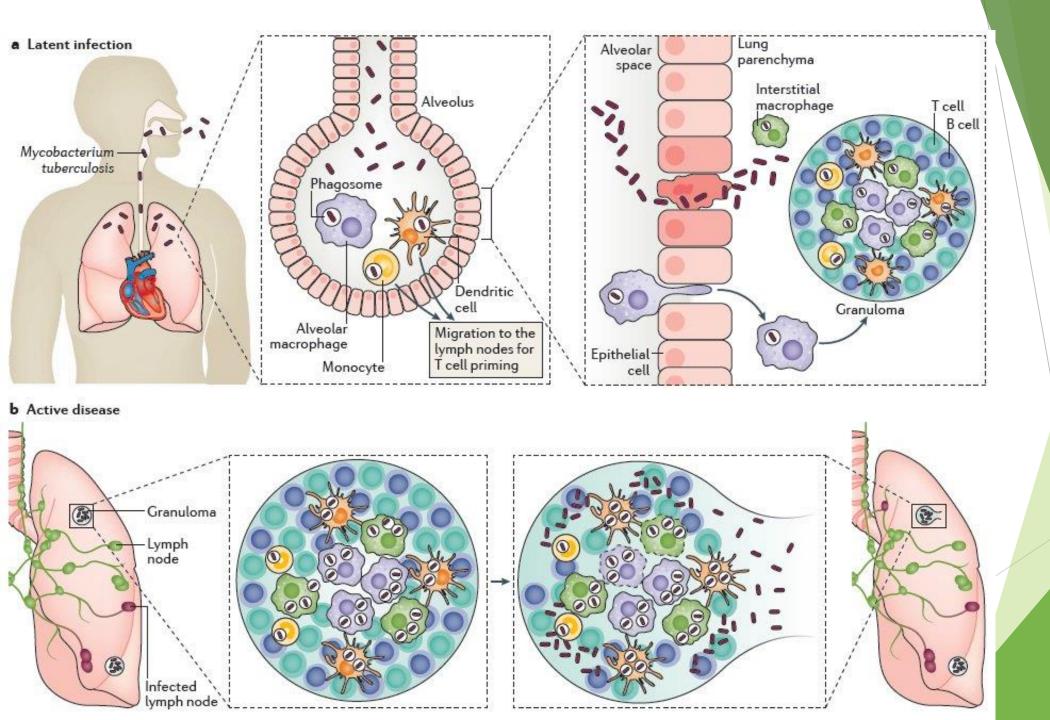
Lungs infected with tuberculosis (TB)

**Healthy lungs** 

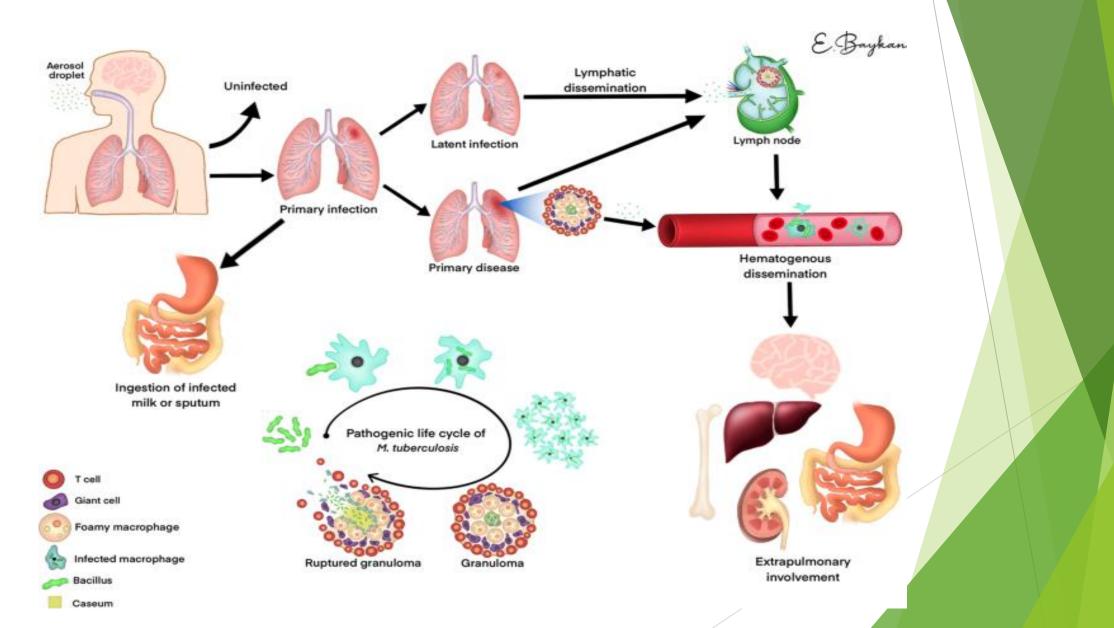
*Cavern* – with a decrease in resistance, granulomas undergo ne rosis and the L-forms of bacteria become virulent. As a result, the process is activated, pathogens penetrate into the surrounding tissues, sometimes forming cavities/ in the lung tissue.



Cavities in lung tissue - cavities

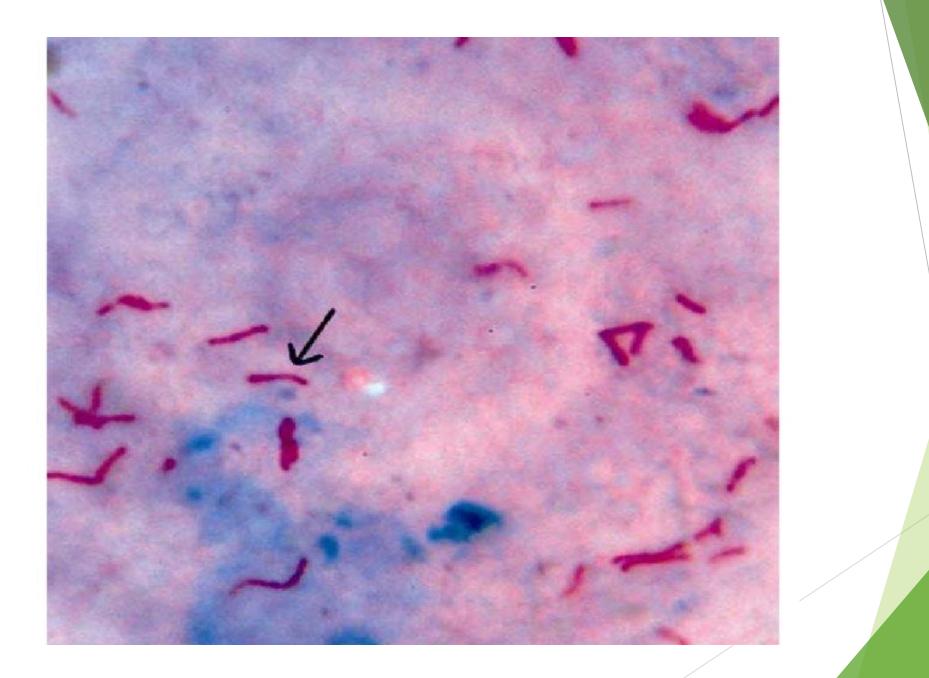


# **Extrapulmonary forms of tuberculosis**



## Microbiological diagnosis of pulmonary tuberculosis

- Bacterioscopic method microscopy, material enrichment (flotation and sedimentation),
- fluorescence microscopy
- Bacteriological method classical (5-6 weeks)
- Price method (microcultures) (1-2 weeks) automated broth culture systems
- biological sample
- Tuberculin diagnostics
- Molecular genetic (PCR)



#### **Microbiological diagnostics:**

- The bacteriological method is the "gold standard", obtaining a culture of the pathogen, its identification and the study of sensitivity to chemotherapeutic drugs.
- The Price microculture method is used for accelerated diagnosis (1-2 weeks).
- Automated cultivation method BACTEC MGIT system is used. Inside the BACTEC culture system, in MGIT (Mycobacteria Growth Indicator Tube) tubes, there is a modified Middlebrook 7H9 nutrient broth.

### Cultivation system BACTEC MGIT



#### **Test tubes** *MGIT* (*Mycobacteria Growth Indicator Tube*)



### **Microbiological diagnostics:**

- Molecular genetic method (PCR) allows you to reduce the time of the study to two days, the sensitivity is 55-90%, the specificity is about 100%.
- The serological method ELISA, is used to detect specific antibodies to the causative agent of tuberculosis in the blood serum. With the help of ELISA, not a disease is determined, but an infection.
- Biological method the detection of tuberculosis pathogens in pathological material is based on the infection of laboratory animals.

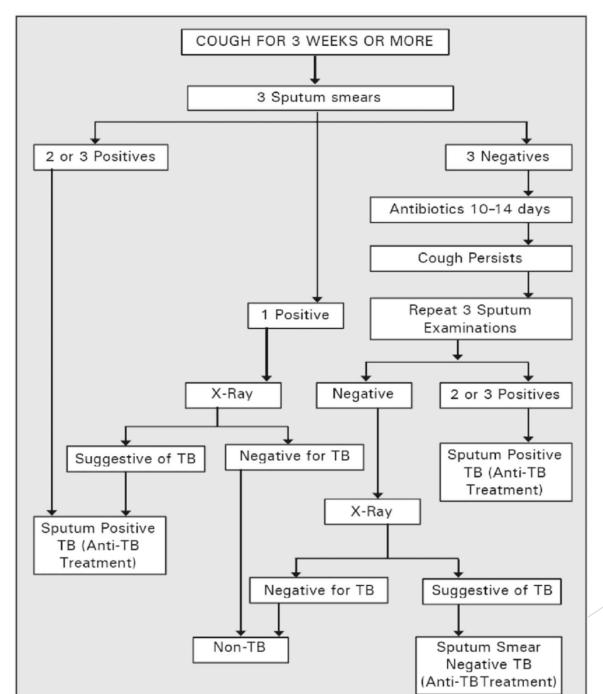
#### **Microbiological diagnostics :**

- ▶ The skin allergy test is based on the reaction of DTH (latent tuberculosis infection) to tuberculin. To do this, a certain dose of tuberculin (PPD) is administered as an intradermal injection on the anterior surface of the forearm (Mantoux test). This test is widely used for mass examination of the population, and timely detection of primary infection (turn) of children and adolescents.
- A positive tuberculin test does not indicate a disease, but an infection.

# **Tuberculin test**



#### DIAGNOSTIC ALGORITHM FOR PULMONARY TB



#### **Determination of the sensitivity of the causative agent of tuberculosis to chemical therapeutic drugs:**

- Exact concentration method based on the cultivation of M.tuberculosis on nutrient media with the addition of antituberculosis drugs in a certain concentration.
- Resazurin test based on the reduction of resazurin and the change in the color of the medium, allows you to more quickly assess the growth of mycobacteria.

**Determination of the sensitivity of the causative agent of tuberculosis to chemical therapeutic drugs:** 

- **Determination of resistance genes using PCR**
- Mutation in the InhA gene encoding mycolic acid enzymes - sensitivity to isoniazid
- Mutation in the rpsL gene encoding a ribosomal protein
  sensitivity to streptomycin
- Sensitivity to rifampicin may be due to changes in subcomponent b in RNA polymerase (mutation in the rpoB gene)

#### **Tuberculosis treatment:**

- First-line drugs: isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin
- Second-line drugs: kanamycin, ethionamide, cycloserine, quinolones (ofloxacin, ciprofloxacin) are more toxic and ineffective
- The current standard of chemotherapy for tuberculosis is treatment with four first-line drugs - a combination of isoniazid, rifampicin, pyrazinamide, ethambutol continuously for 6-9 months.

#### **Prevention of tuberculosis:**

- Specific prophylaxis carried out with the BCG vaccine (Bacillus Calmette-Guerin)
- The BCG vaccine is given to newborns in the first week of life as an intradermal injection.
- Revaccination at 7 and 12 years, then up to 30 years every 5-6 years.
- Premature babies are immunized with a weakly reactogenic BCG-M vaccine, in which the amount of bacteria is 2 times less.

#### mycobacteria pathogenic for humans and their properties

Kind	Tank	Pathogenici ty for humans	The diseases they cause	Person to person transmission
M.tuberculosis	human	+ + +	tuberculosis	yes
M.bovis	animals	+++	tuberculosis	In rare cases
M.kansasii	Environment objects	+	Tuberculosis Similar Lesions	In very rare cases
M.scorfulaceum	Environment objects	+	lymphadenitis	no
M.avium- intracellulare	Environmental objects, birds	+	Tuberculosis Similar Lesions	no
M.fortuitum	Environment objects	+	Skin abscesses	no
M.marinum	water, fish	±	Skin granulomas	no
<b>M.ulcerans</b>	Environment objects	±	Skin ulcers	no
M.leprae	human	+ + +	leprosy	yes

# Causative agents of mycobacteriosis

## **Runyon classification**

I Group	slow growing photochromogenic	M.kansasii, M.marinum	
II Group	slow growing scotochromogenic	M.scorfulaceum, M.gordonae	
III Group	slow growing achromogenic	M.avium complex (M.avium,M.intracellulare) M.xenopi, M.ulserans-(язва Бурули)	
IV Group	fast growing	M.fortuitum, M.phlei, M.smegmatis, M.chelonei	

#### Mycobacterium leprae specimen from lepromatous nodules (Ziehl-Neelsen stain)



# **BIOLOGICAL PROPERTIES**

- Obligate intracellular parasites (reproduce in the body of nine-banded armadillos, which have a low body temperature)
- Possess tropism for tissues with low temperature (skin, nasal mucosa, superficial peripheral nerves, binding to the G domain of the basal layer of Schwann cells)
- **Found in the cytoplasm of cells resembling cigars in packs**

# PATHOGENIC FACTORS

- **Fibronectin-binding protein promotes entry into cells**
- Microcapsule and cell wall lipids provide resistance to lysosomal enzymes
- **Wax (leprosin and leprosic acid)**
- **The enzyme O-diphenol oxidase is involved in skin hypopigmentation**

# **MYCOBACTERIUM LEPRAE**

Mycobacterium leprae (also known as the leprosy bacillus or Hansen's bacillus), is a bacterium that causes Hansen's disease (leprosy), which is a chronic, curable infectious disease that damages the peripheral nerves and targets the skin, eyes, nose, and muscles. Leprosy can occur at all ages from infancy to old age. It was discovered in 1873 by the Norwegian physician Gerhard Armauer Hansen. It was the first bacterium to be identified as causing disease in humans

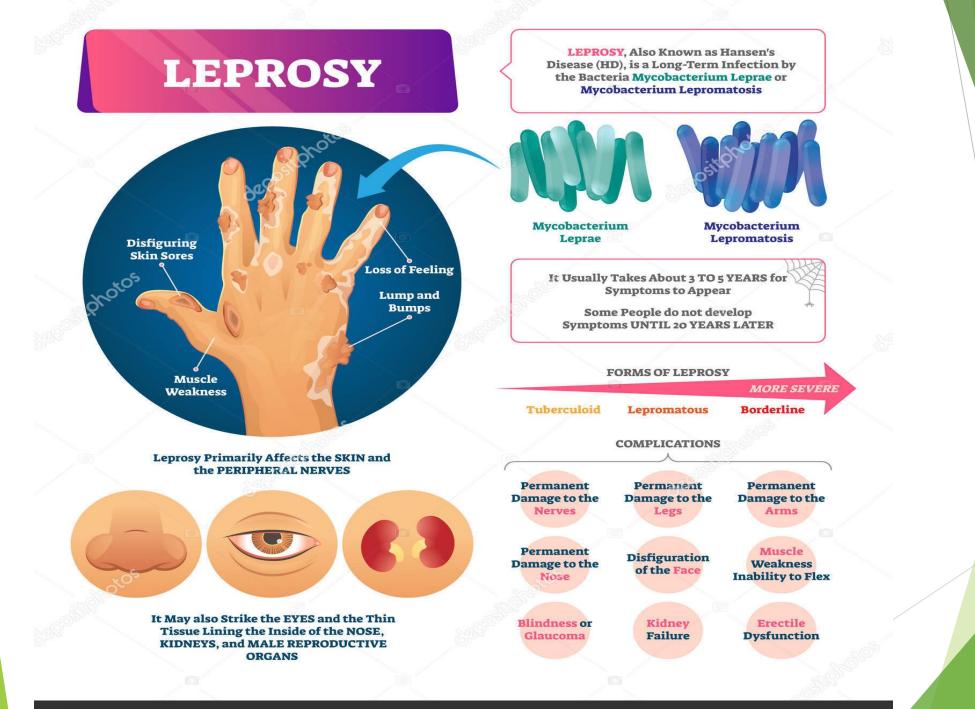
## LEPROSY





The disease is divided into two major types

- Leproma- tous and
- Tuberculoid, with several intermediate stages (see the Ridley-Jopling classification system).



# DİAGNOSİS

Scrapings with a scalpel blade from skin or nasal mucosa or from a biopsy of earlobe skin are smeared on a slide and stained by the Ziehl-Neelsen technique. Biopsy of skin or of a thickened nerve gives a typical histologic picture. No serologic tests are of value. Nontreponemal serologic tests for syphilis frequently yield false-positive results in patients with leprosy.

Received at Microbiology lab Modified Acid-Fast stain Positive Negative (BI measured) Slides cleaned with xylene. Slides scrapped with fine needle. DNA Extraction. PCR Results obtained, compared with SSS results

Slit-skin smear processed from Dermatology Department

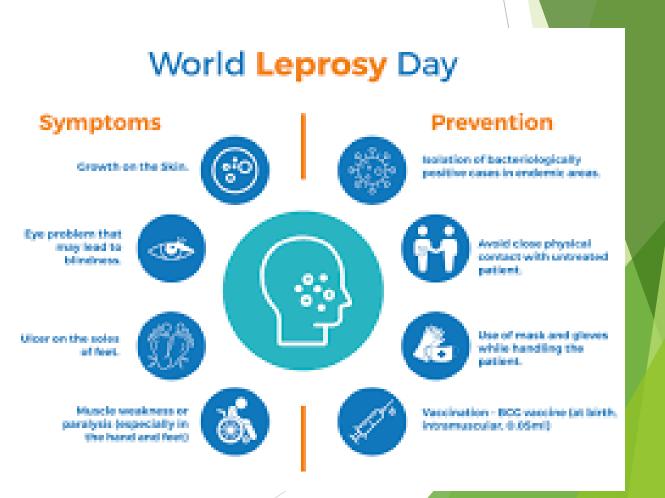
## **DIAGNOSTICS OF LEPROA**

- Bacterioscopic examination (tissue fluid, sputum, lymph nodes, biopsy specimens) - Ziehl-Neelsen staining
- Serological diagnostics detection of antibodies to phenolic glycolipid using ELISA
- **PCR to detect leprosy antigens in tissues**
- KA test with lepromin With intradermal administration of lepromin, after 48 hours, the Fernandez reaction may develop, and after 3-4 weeks, the Mitsuda reaction (tubercle, node, sometimes necrosis).

# PREVENTATION

#### PREVENTION

- Avoiding physical contact with untreated people
- People who are in immediate contact with the leprosy patient should be tested for leprosy.
- Annual examinations



## Actinomycetes

- Gram-positive branching filamentous or rod-shaped bacteria
- Like fungi, they form a mycelium consisting of intertwining thin filaments (hyphae), but unlike fungi, they do not contain chitin or cellulose in the cell wall.
- ▶ In the affected tissues form drusen.
- Able to reproduce by fragmentation of the mycelium into rod-shaped and coccal forms, as well as by the formation of spores

## **Taxonomy of actinomycetes**

- Type Actinobacteria
- Class Actinobacteria
- Genus Actinomyces
- Species A.israelii, A.bovis, A. odontolyticus, A.viscosus,
  A.naeslundii (causative agents of actinomycosis)

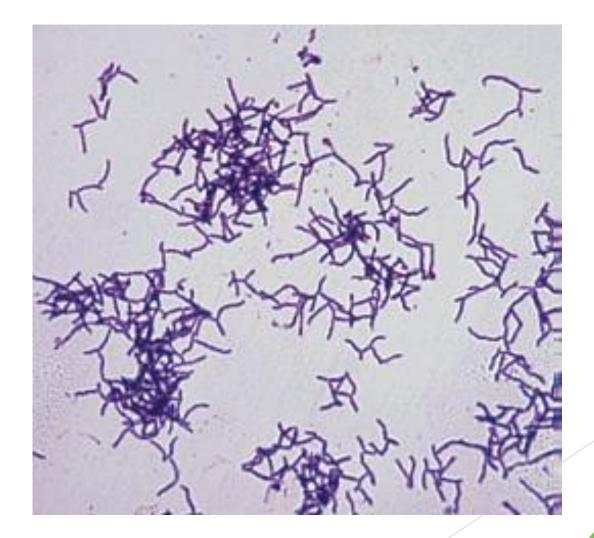
#### **Differential characters of representatives of the genus Actinomyces**

ORGANISM	ACID FROM CELLOBIOSE	HYDROLYSIS OF					LYSOZYME
		CASEIN	HYPOXANTHINE	TYROSINE	UREA	XANTHINE	RESISTANCE
Actinomadura madurae	+	+	+	+	_	:	-
A. pelletieri		+	+/-	+	-		20 <del>11-</del> 20
Nocardia as- teroides		—	-	-	+	°	+
N. brasiliensis	—	+	+	+	+		+
N. caviae	-	-	+	+/-	+	+	+
Nocardiopsis dassonvillei	+	+	+	+	+/-	+	-
Streptomyces griseus	+	+	+	+	+	+	-
S. somaliensis		+	-	+		—	
Streptomyces species (other)	+/-	+	+	+	+/-	+/-	+/-

 $= \ge 90\%$  of strains positive;  $- = \ge 90\%$  of strains negative; +/- = variable results.

odified from McGinnis et al. 1982. Pictorial handbook of medically important fungi and aerobic actinomycetes. Praeger Publishers, w York.

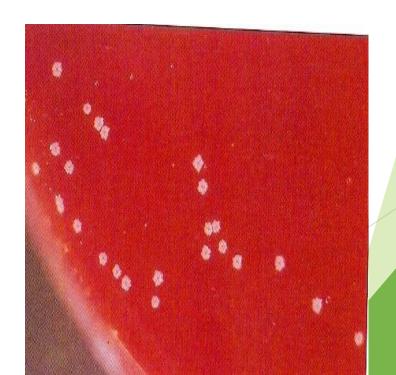
# Type species of Actinomyces bovis in a smear from a pure culture

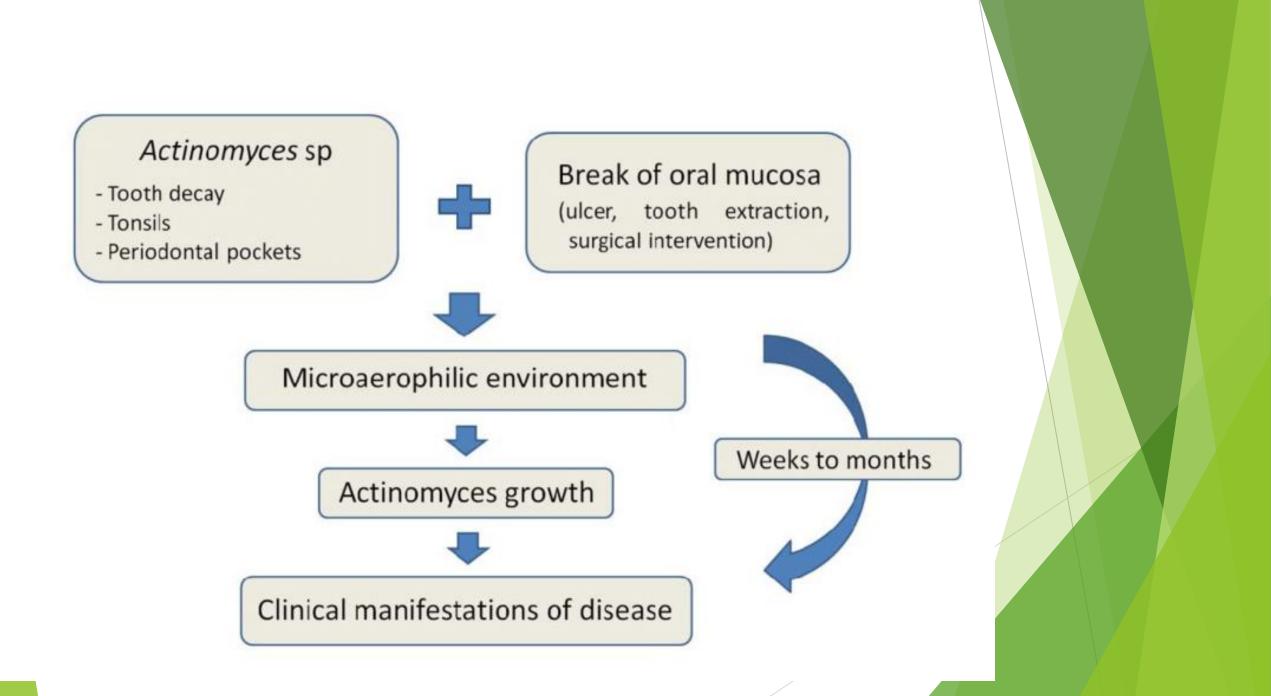


## **Colony of Actinomyces**

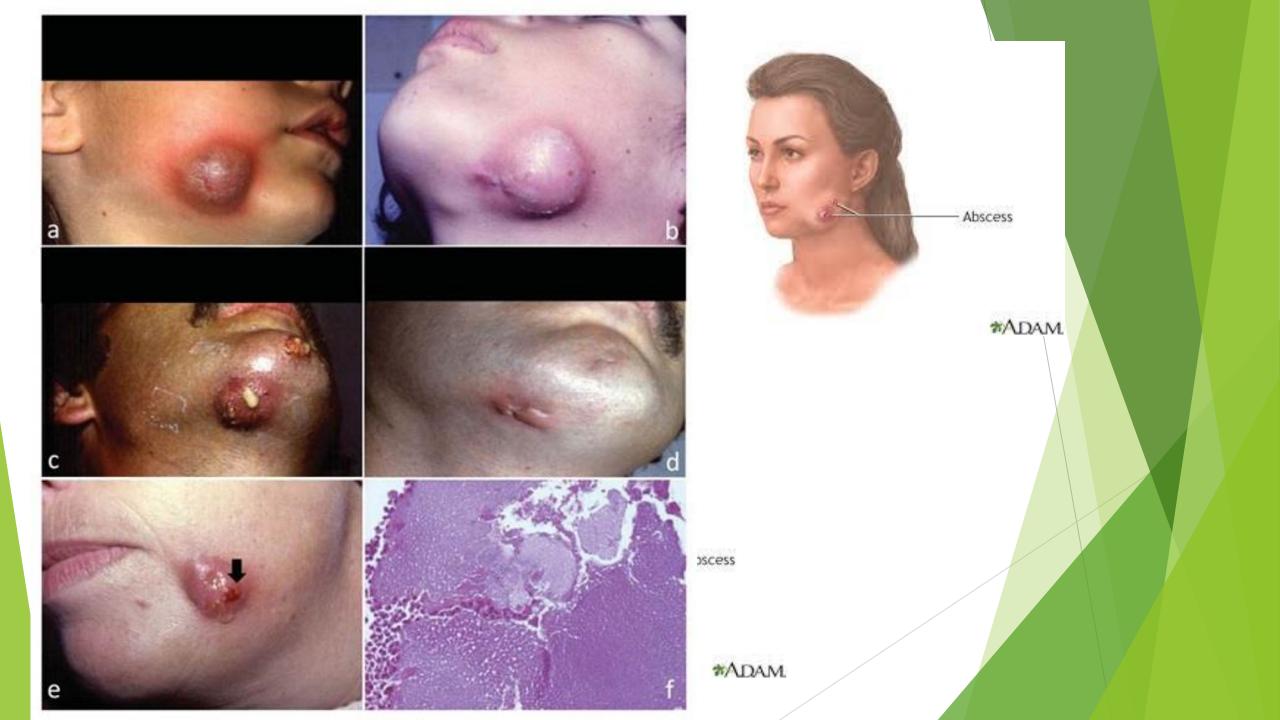
grow on media containing hemin, vitamin K, 5% CO2, thioglycol medium, heart-brain broth. Colonies resemble molars, loose pasty or smooth, colorless



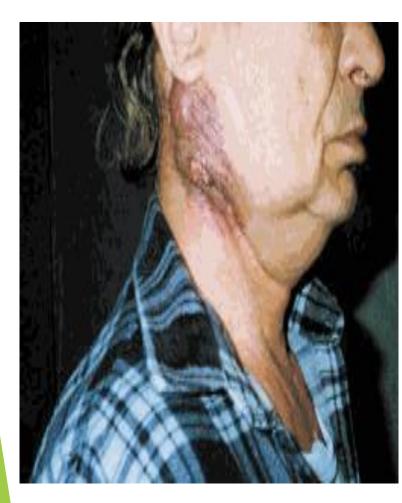


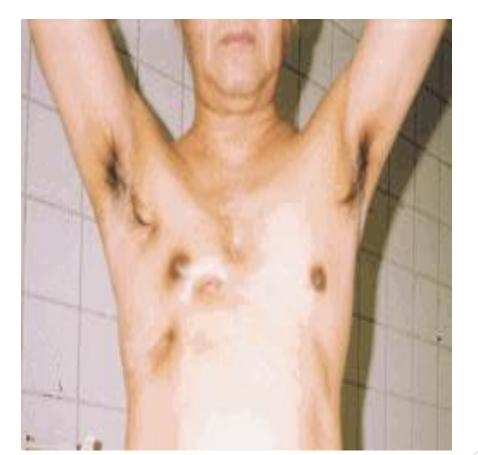


Actinomycosis is a chronic opportunistic infection characterized by granulomatous inflammation with polymorphic clinical manifestations. The most frequently encountered pathogens: A.israelii A. bovis A. odontolyticus A.viscosus A.naeslundii



## Actinomycosis





## Actinomycosis of the face

- Most lesions are caused by saprophytic bacteria that live in the oral cavity, especially in the cavity of carious teeth, in deposits of tartar.
- Actinomycosis develops only against the background of a decrease in resistance as a result of beriberi, severe diseases, etc.
- Actinomycosis of the cervicofacial region and lower jaw is most often observed. The causative agent overcomes the epithelial barrier of the oral mucosa during injuries, surgical interventions, injections

## actinomycosis of the face

- In the mucous membrane or in deep soft tissues, one, and more often several dense nodes-granulomas (actinomycomas) develop without acute inflammatory phenomena, fever and impaired health.
- Signs of intoxication with headaches, general weakness and subfebrile body temperature appear only when the nodes disintegrate with the release of pus through several narrow fistulas.
- With the localization of nodes in the lower jaw, convulsive spasm of the muscles of the mouth (trismus) often develops, making it difficult to eat.

## actinomycosis of the face

Подкожная форма актиномикоза щечной области слева



АКТИНОМИКОЗ СЛИЗИСТОЙ ЩЕКИ СПРАВА



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#### ПОДКОЖНАЯ ФОРМА АКТИНОМИКОЗА



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#### АКТИНОМИКОЗ ЛИЦА СЛЕВА

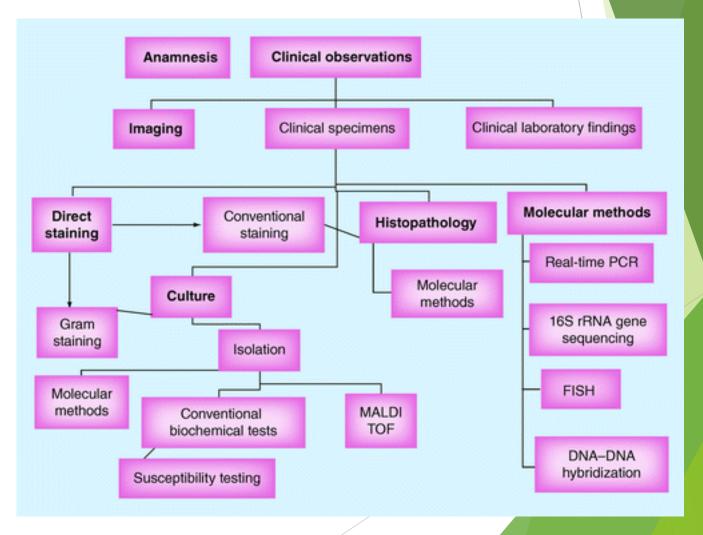


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#### LABORATORY DIAGNOSIS

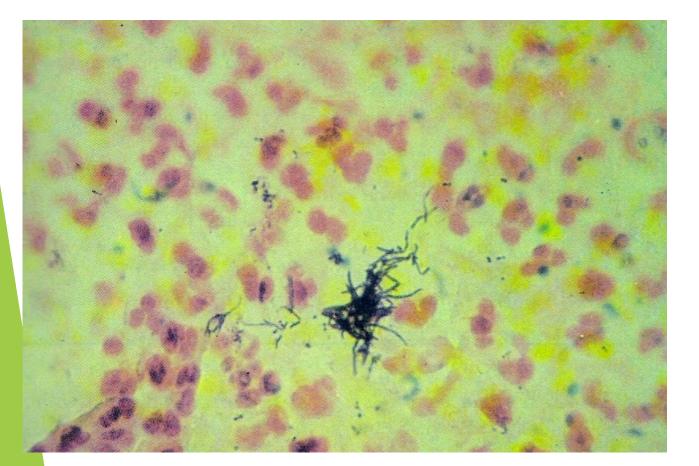
#### Laboratory diagnosis

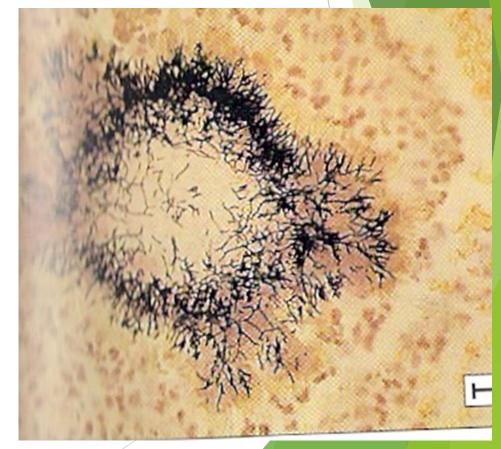
- Specimens pus, sputum, tissue biopsy
- Microscopic examination sulphur granules
- Culture thioglycolate medium and BHI
- · Incubation: anaerobic for 2 weeks
- Gas liquid chromtography (GLC) of metabolic by-products



## Actinomyces israelii

pus preparation





#### Drusen in pathological material

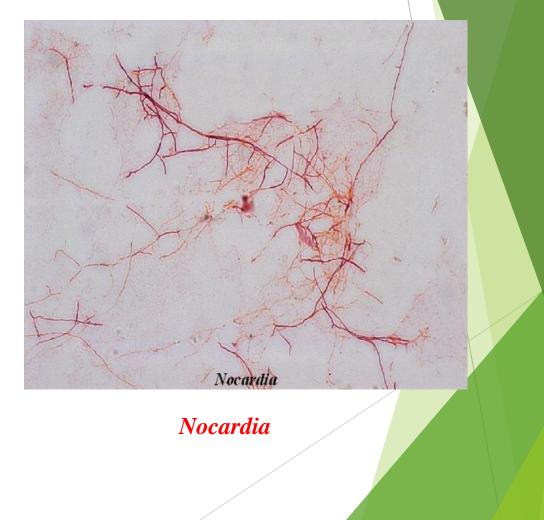


## Failure

- Bacteria of the genus Nocardia cause nocardiosis in humans.
- Nocardiosis is often caused by the Nocardia asteroides complex (Nocardia farcinica, Nocardia nova, Nocardia abscessus, and other species), in rare cases by Nocardia brasiliensis and Nocardia otitidiscaviarum species, and in very rare cases by other species.

#### Nocardia asteroides Morphological properties:

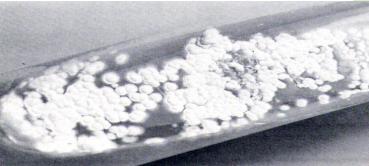
*Nocardia* – These are rod-shaped bacteria with highly branched aerial and substrate mycelium. In the first hours, the mycelium is divided into segments and consists of one cell. Gradually, mycelia, forming partitions (septa) are divided into rod-shaped and coccal elements. They are Gram-positive and partially acid-fast.



#### Nocardia asteroides

It develops on ordinary nutrient media at a temperature of 28-370C under aerobic conditions. On nutrient media, after a few days or a week, they form glomerular waxy colonies. Synthesizes and releases into the nutrient medium a pigment from white to pink, even red.





waxy colonies

## Nocardia

- **Source of infection and causative agent:**
- The source of infection is the soil.
- N.asteroides mainly enters the body by air-dust (aerogenic) way and causes a chronic infection of the lungs.
- N.brasiliensis infection occurs when soil enters through damaged skin during injuries, resulting in subcutaneous lesions.

## Nocardiosis

- Treatment with corticosteroids, organ transplantation, tuberculosis can contribute to the development of nocardiosis.
- ▶ The main forms include nocardiosis of the skin and lungs.
- The most common are pulmonary nocardiosis caused by Nocardia asteroides

AIDS,

 nocardiosis of the skin caused by Nocardia brasiliensis and leading to subcutaneous lesions

#### **Nocardiosis** Nocardia brasiliensis infection



## **Microbiological diagnostics:**

- Material for research:
- sputum
- pus
- liquor
- tissue biopsy
- Microscopic method gram-positive coccobacilli are detected, as well as branched filaments.
- Bacteriological method obtaining a culture of the pathogen

## **Treatment :**

- The drug of choice is sulfamethoxazole-trimethoprim (biseptol)
- If treatment with biseptol is ineffective, the antibiotics amikacin, imipinem, cefotaxime and other antibiotics are used.
- Since some antibiotics do not penetrate into abscess cavities and granulomas, in some cases surgery is required.

## **Actinomycetes pathogens:**

 Actinomycetoma is a type of mycetoma and is formed by infection mainly with actinomycetes Nocardia asteroides, Nocardia brasiliensis, Streptomyces somaliensis and Actinomadura madurae.

The causative agents of actinomycetoma live in the soil and on the surface of plants..

### Actinomycetoma

The pathogen enters the body through broken skin. Gradually formed papule, deep nodes and abscesses.

The destructive process extends to the fascia, muscles and bones. Fibrinous tissue develops. The foot swells and the process, penetrating into the bones, leads to foot deformity. From the fistulas that open to the surface of the skin, pus is released.



Actinomycetoma

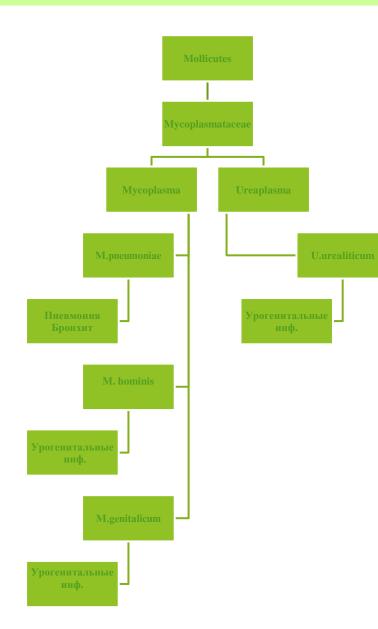
#### **Microbiological diagnosis and treatment:**

Microscopic method - based on the detection of drusen and branched actinomycetes in pus and biopsy specimens.

**Treatment:** 

- streptomycin,
- combination of sulfamethoxazole-trimethoprim and dapsone
  - surgical intervention (amputation of the affected limb)

## Mycoplasmas and the diseases they cause



## Genus Mycoplasma

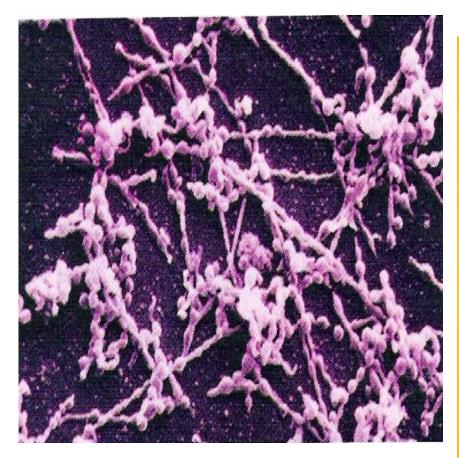
- The smallest free-living bacteria that do not have a cell wall and are surrounded only by a cytoplasmic membrane.
- They are membrane parasites, attaching to the host cell, can multiply for a long time and persist in the body, changing the metabolism of infected cells.

## **Mycoplasma properties**

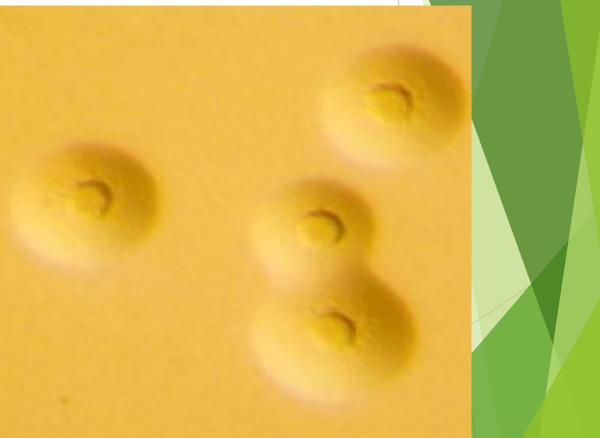
The absence of a cell wall causes polymorphism of mycoplasmas, osmotic sensitivity, the ability to pass through bacterial filters, and resistance to drugs that inhibit cell wall synthesis.

They have a rod-shaped, coccoid, filamentous, branching shape. Gr (-), capsules, spores do not form

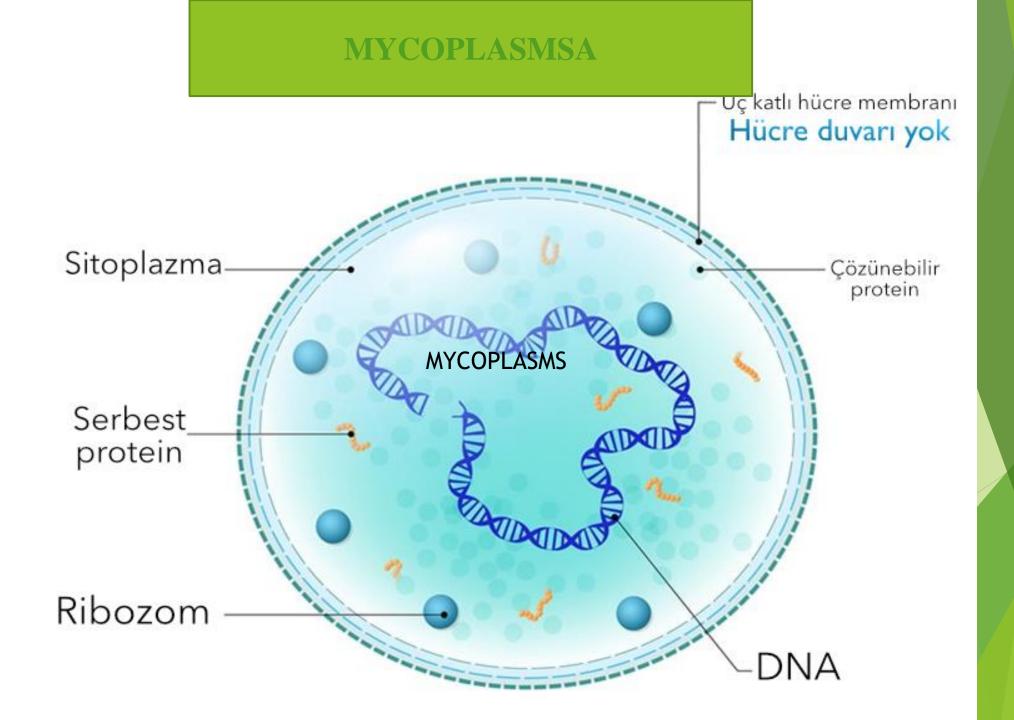
## Mycoplasmas



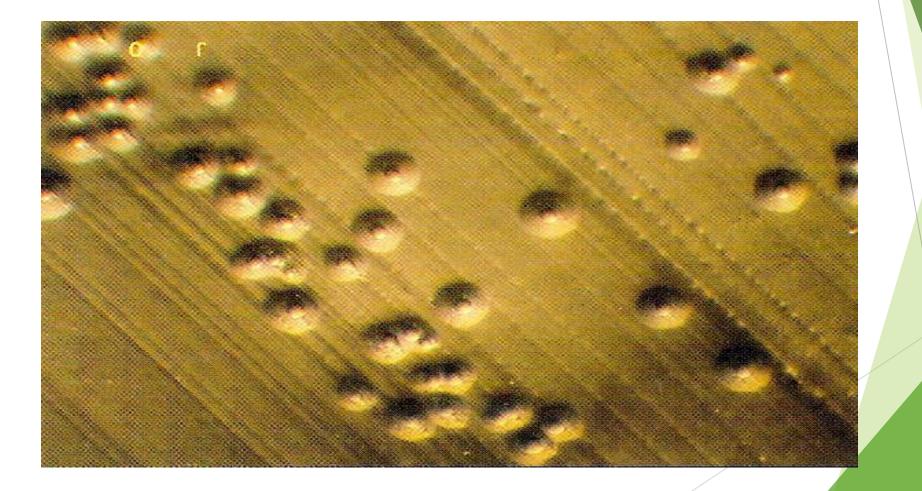
*mycoplasmas under the electron microscope* 

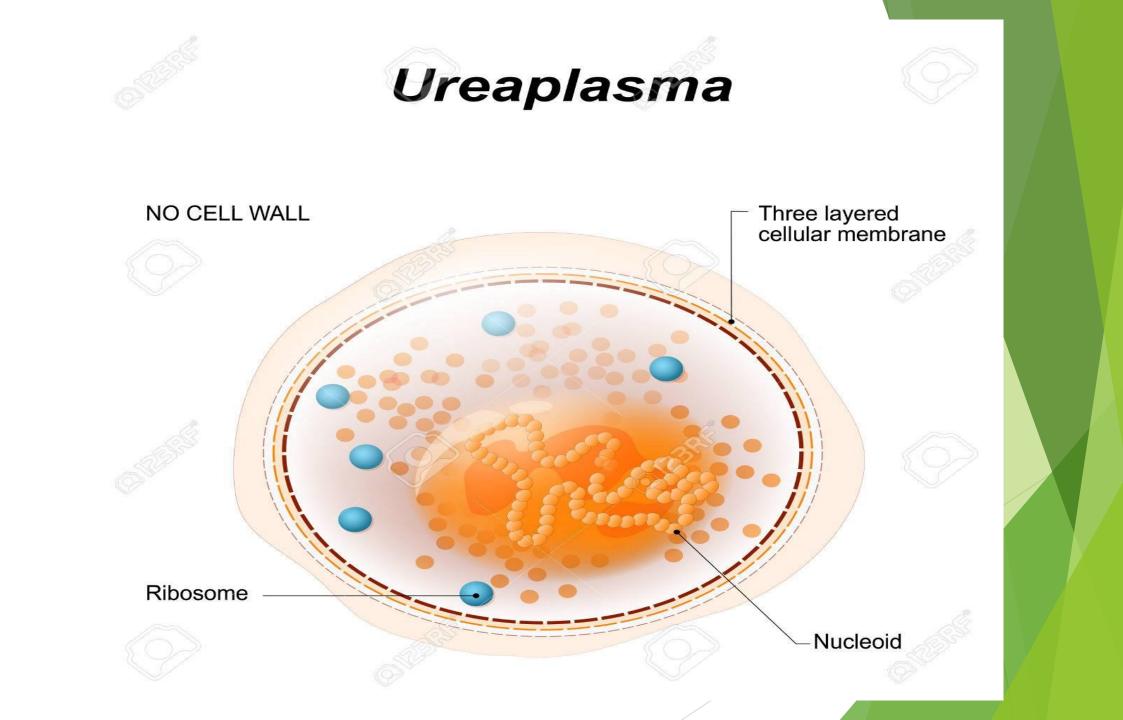


mycoplasma colonies

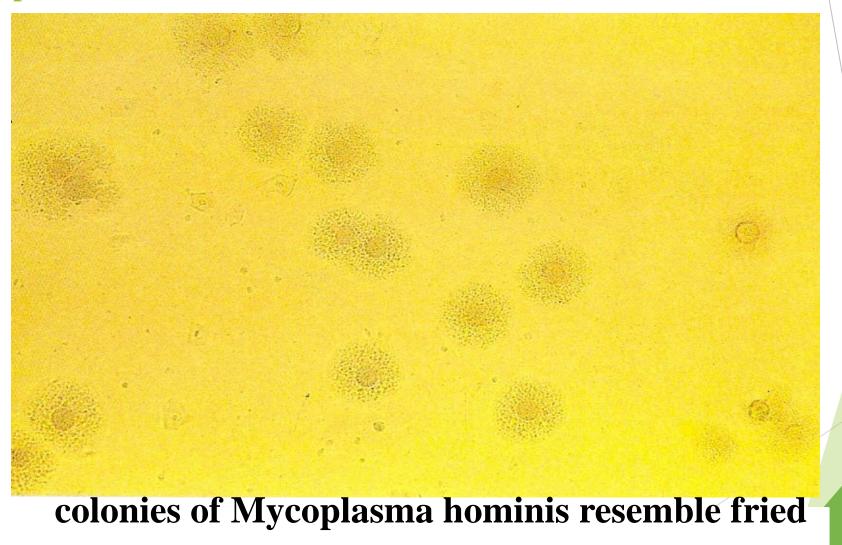


## Mycoplasma hominis (colonies on agar)





Grow on complex media containing extracts of yeast, heart and be tissue, serum, sterols, carbohydrates. Cultivation on KK and in RK possible



eggs

> Pathogenic mycoplasmas are flask-shaped and filamentous in shape with a specific polar terminal structure that allows the microbe to attach to the host cell. This structure is represented by a complex of adhesin proteins and adhesin-accessory proteins rich in proline, which provides protein folding, which is necessary for attachment to the cell. Mycoplasmas attach to the host cell membrane via glycolipid receptors. The formation of hydrogen peroxide and peroxide radicals causes damage to the host cell membrane, resulting in tighter membrane fusion upon attachment of mycoplasmas. In this case, the protective function of the ciliated epithelium is suppressed. Mycoplasmas are membrane parasites, attaching to the host cell, they can multiply and persist in the macroorganism for a long time, changing the metabolism of infected cells, exerting a cytotoxic and cytolytic effect on them.

## ЗАБОЛЕВАНИЯ, ВЫЗЫВАЕМЫЕ МИКОПЛАЗМАМИ

- M.pneumoniae является возбудителем респираторного микоплазмоза (первичной атипичной пневмонии);
- Ureaplasma urealyticum, M.hominis, M.genitalium являются причиной урогенитальных заболеваний, патологии плода и новорожденных;
- M.arthritidis и др. поражений суставов (ревматоидный артрит).

### Патогенные представители родов Mycoplasma и Ureaplasma

Вид	Болезнь
M.pneumoniae	Воспаление верхних дыхательных путей, трахеобронхит, атипичная пневмония
M.hominis	Пиелонефрит, воспалительные заболевания тазовых органов, послеродовая лихорадка, пороки развития
M.genitalium	Негонококковый уретрит (урогенитальный микоплазмоз)
M.fermentans	Воспалительные заболевания респиратор- ного тракта, ревматоидный артрит
<b>U.urealyticum</b>	Негонококковый уретрит, хронические заболевания лёгких, врождённые пневмонии, бесплодие

## ΠΑΤΟΓΕΗΕ3

- Возбудитель попадает на поверхность слизистых дыхательных путей, и прикрепляется к мембране эпителиальных клеток.
- Происходит встраивание участков мембраны возбудителя в мембрану клеток и вследствие чего внутриклеточное паразитирование микоплазм.
- Пневмония, вызываемая *M. pneumoniae*, нередко интерстициальная (инфильтрация и утолщение межальвеолярных перегородок, появление в них лимфоидных гистиоцитарных и плазматических клеток, поражение альвеолярного эпителия).
- Увеличение перибронхиальных лимфатических узлов.
- иммунопатологические реакции (видимо, обусловливающим внелёгочные проявления микоплазмоза).
- Для респираторного микоплазмоза характерно образование холодовых агглютининов, активация В- и Т-лимфоцитов и повышение уровня общего сывороточного IgM.

#### КЛИНИЧЕСКАЯ КАРТИНА РЕСПИРАТОРНОГО МИКОПЛАЗМОЗА

- Воспалительная инфекция верхних дыхательных путей (фарингит, трахеобронхит) - заболевание сравнительно легкое, чаще субклиническое.
- Проявляется как назофарингит, трахеит, бронхит.
  Инкубационный период от 3 до 11 дней, хотя может быть до 3-х недель.
- Температура тела 37,1-37,5°С, реже 38°С.
- Кашель малопродуктивный. Чаще инфекция благополучно разрешается, но может распространиться на легкие.

Возможна гематогенная диссеминация в суставы, костный мозг, мозговые оболочки, развитие иммунопатологических процессов. Особенностью заболевания является относительная слабая контагиозность, высокая частота бессимптомных и легких форма

# M. hominis, U.urealyticum, M.genitalium - causative agents of urogenital mycoplasmosis

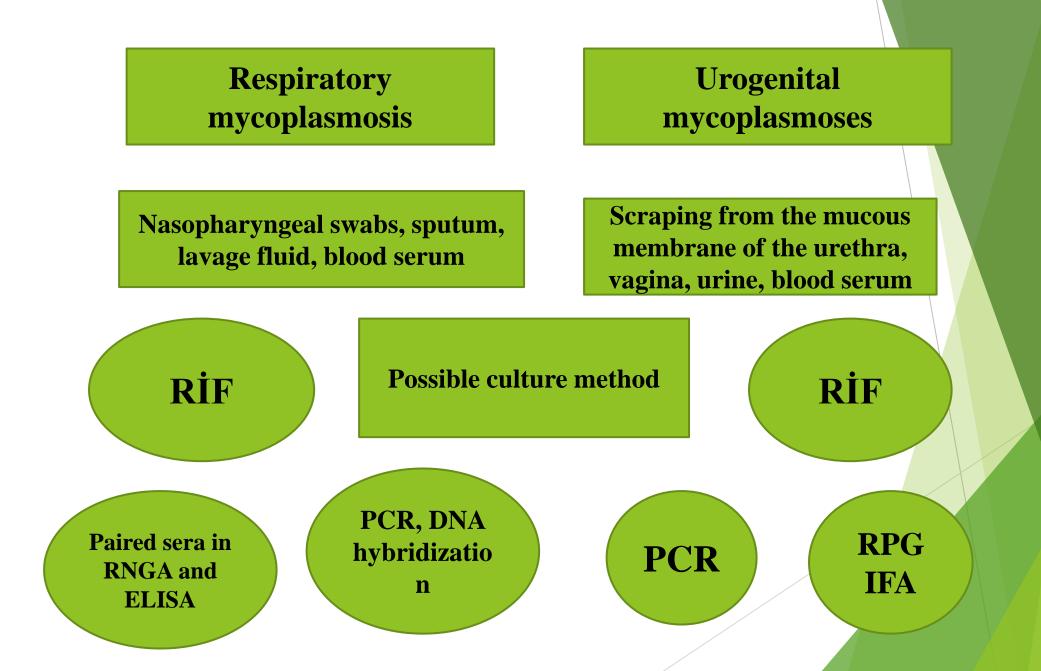
- **The source of infection is a sick person.**
- M.genitalium causes non-gonococcal urethritis in men, in women cervicitis, endometritis, salpingitis
- **U.urealyticum causes non-gonococcal urethritis in men**
- M. hominis causes salpingitis in women, as well as postpartum and post-abortion fevers
- M. hominis and U.urealyticum can infect the fetus during childbirth and cause pneumonia in newborns

#### ОПОРНЫЕ КЛИНИЧЕСКИЕ СИМПТОМЫ ХЛАМИДИЙНО-МИКОПЛАЗМЕННОЙ ИНФЕКЦИИ

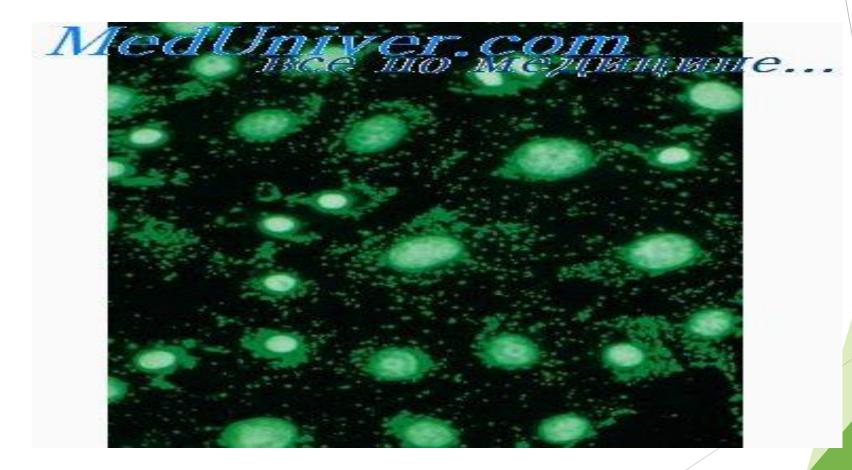
- Постепенное начало
- Длительный субфебрилитет

Последовательное возникновение признаков поражения бронхо-легочной системы: коньюнктивит, ринофарингит, бронхит, атипичная малосимптомная пневмония

- Упорный приступообразный мучительный кашель
- Лимфаденопатия
- Длительное течение заболевания



## Mycoplasmas - membrane parasites (detection with RIF)



#### Mycoplasma pneumoniae (colonies)

