AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

LESSON 20.

Introduction to clinical microbiology. Healthcare-associated infections.

Microbiological diagnosis of infections of the respiratory tract, gastrointestinal tract and microbiota disorders

FACULTY: ***General Medicine***
SUBJECT: ***Medical microbiology - 2***

Discussed questions:

1. Understanding of clinical microbiology.

2. Infections related to medical care, causes of occurrence, clinical forms, characteristics of causative agents. Infection control.

3. Upper and lower respiratory tract, brief anatomical and physiological information

4. Normal microflora of the upper respiratory tract, inflammatory diseases and their causative agents, rules for taking pathological material, microbiological diagnosis

5. Inflammatory diseases of the lower respiratory tract, their types, causes.

6. Principles of microbiological diagnosis of sputum.

7. Microscopic and bacteriological examination of sputum.

8. Examination of other pathological materials (bronchi content, trachea and lung tissue punctate and aspirate, pleural fluid).

9. Gastrointestinal tract, brief anatomical and physiological information

10. Normal microflora of the gastrointestinal tract, concepts of dysbiosis and dysbacteriosis

11. Inflammatory diseases of the gastrointestinal tract and their causes.

12. Rules for obtaining pathological material.

13. Principles of microbiological diagnosis of gastrointestinal infections.

14. Microbiological examination of feces and diagnostic indicators.

15. Criteria and microbiological diagnosis of microbiota disorders

Purpose of the lesson:

- To acquaint students with the goals and objectives of clinical microbiology. To provide them with information about infections related to medical care, to teach the agents of normal microflora and inflammatory diseases of the upper and lower respiratory tract, principles of diagnosis of these diseases. To provide students with information about the normal microflora of the gastrointestinal tract, the concepts of dysbiosis and dysbacteriosis, inflammatory diseases of the gastrointestinal tract, to introduce them to the causes of gastrointestinal tract diseases and the principles of disease diagnosis

Clinical microbiology

- Clinical microbiology studies the microbiology of diseases of organs and systems of the human body, the principles of their microbiological diagnosis.

The microbiology and diagnostics principles of the nosocomial infections

Nosocomial infections

- Nosocomial infections (Greek, "nosocomion" - hospital) infections usually develop 48 hours after admission to the hospital and sources of infection may be carriers, contaminated medical instruments and equipment, medical personnel, or people visiting patients.
- Nosocomial infections are common in hospitals and, combined with the underlying disease, exacerbate its course.
- They can be caused by both pathogenic and opportunistic microorganisms.

The causative agents of nosocomial infections

- The causative agents of nosocomial infections are often opportunistic microorganisms.
- The range of pathogens of nosocomial infections is very wide, they are caused by various viruses, bacteria, fungi and protozoa.
- Among the causative bacteria are staphylococci, enterococci, pneumococci, enterobacteria, P. aeruginosa and other non-fermenting bacteria, anaerobes.
- Recently, there has been an increase in nosocomial infections caused by respiratory viruses and Candida fungi.

Characteristics of nosocomial infections

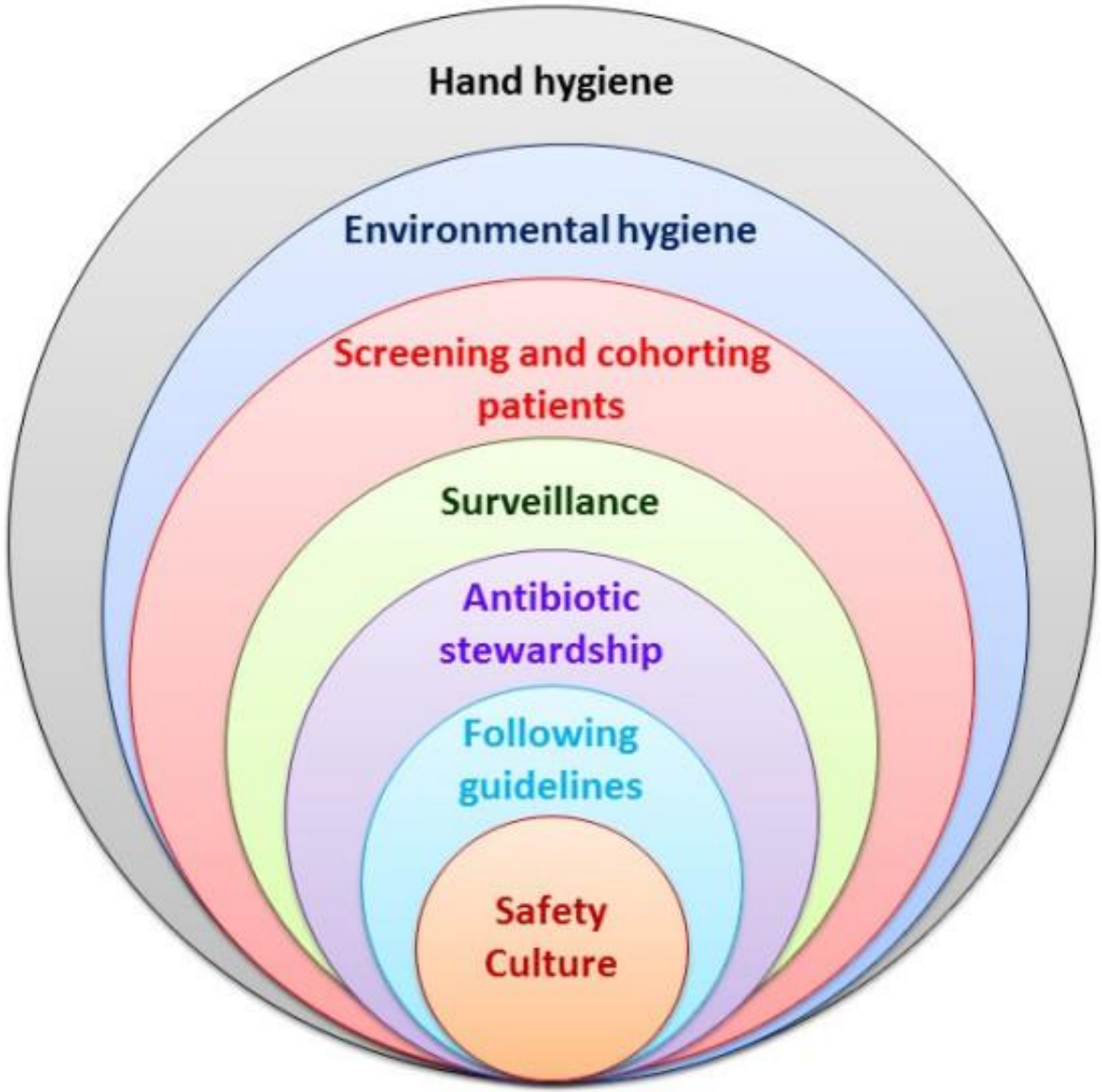
Opportunistic microorganisms that cause nosocomial infections differ in the following features:

- The causative agents of nosocomial infections are more resistant to antibacterial drugs, antiseptics, disinfectants, physical agents, bacteriophages and bacteriocins.
- Bacteria obtaining during nosocomial infections as a rule have higher virulence.
- Since opportunistic microorganisms do not have organ tropism, they can cause diseases in any organ and tissue of the body
- Heterogeneity (antigen variability, etc.) in the population of nosocomial infections is higher than in other microorganisms.

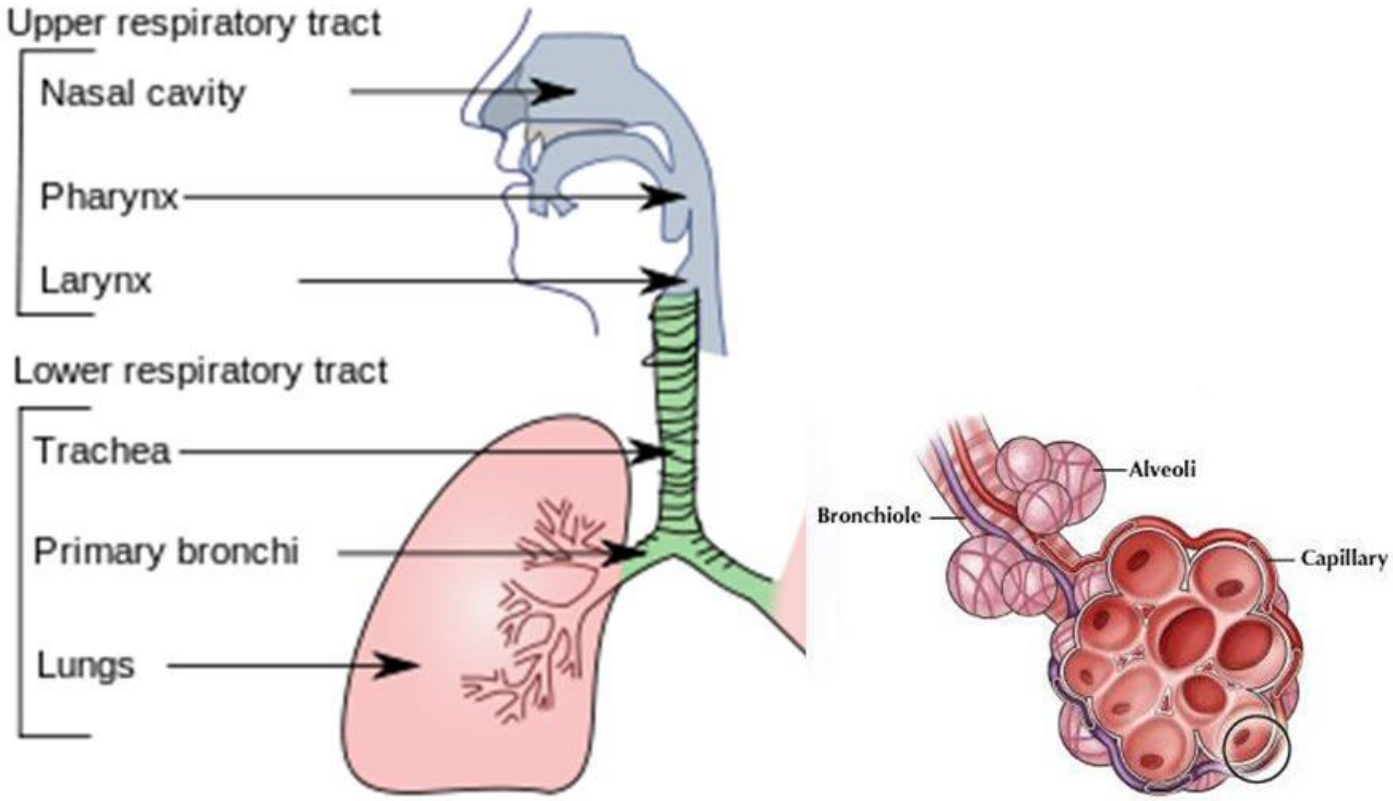
Principles of microbiological diagnosis of nosocomial infections

- Materials for investigation are selected depending on the location and nature of the disease.
- **The microscopic method** allows to make approximate conclusions about the nature of the causative agents and to determine the direction of cultural methods. Depending on the nature of the test material, the smears are then microscopic after staining with appropriate methods.
- **The culture method** is the main method of microbiological diagnosis in nosocomial infections. It is important to determine the sensitivity of the culture to antibiotics and other antimicrobial chemical therapeutic drugs.

7 strategies to prevent healthcare-associated infections



Principles of microbiology and diagnosis of respiratory infections



Upper respiratory tract (anatomy and normal microflora)

- Inflammatory diseases of the upper respiratory tract - pharynx and larynx are often caused by pathogenic microorganisms.
- Nasal mucosa encountered non-patogenic corynebacteria, coagulasa-negative staphylococci, alpha-hemolytic streptococci, Neisseria, sometimes potential pathogen bacterias such as S.aureus, beta-hemolytic streptococci, S.pneumoniae, E.coli, Proteus species.

Microbiology of upper respiratory tract infections

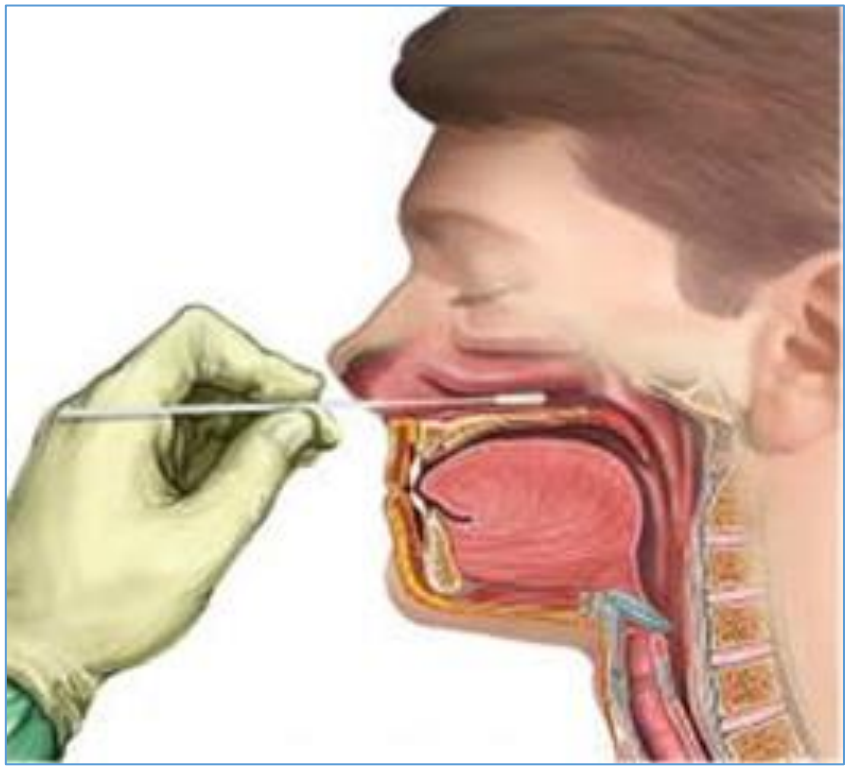
- **Rhinitis and sinusitis** (haimoritis, ethmoiditis, etc.) - adenoviruses, rhinoviruses, coronaviruses, etc.
- **Pharyngitis or angina** (refers to inflammation of the pharynx, soft palate and pharynx) and tonsillitis (inflammation of the tonsils)
- **Catarrhal pharyngitis** - ortho- and paramyxovirus, adenovirus, coronaviruses, herpes simplex virus and Coxsackie virus
- **Purulent pharyngitis** - about 90% caused by S. pyogenes, in other cases by other bacteria, especially S. aureus, S. pneumoniae, C. diphtheria, B. pertussis, H. influenzae, etc.
- **Nasopharyngitis** - N. meningitidis, other bacteria of the genus Neisseria
- **Laryngitis** - parainfluenza virus, C. diphtheria, etc.

Principles of microbiological diagnosis of upper respiratory tract infections

- The specimen for microbiological diagnosis in upper respiratory tract diseases are obtained mainly by **sterile cotton swab**.

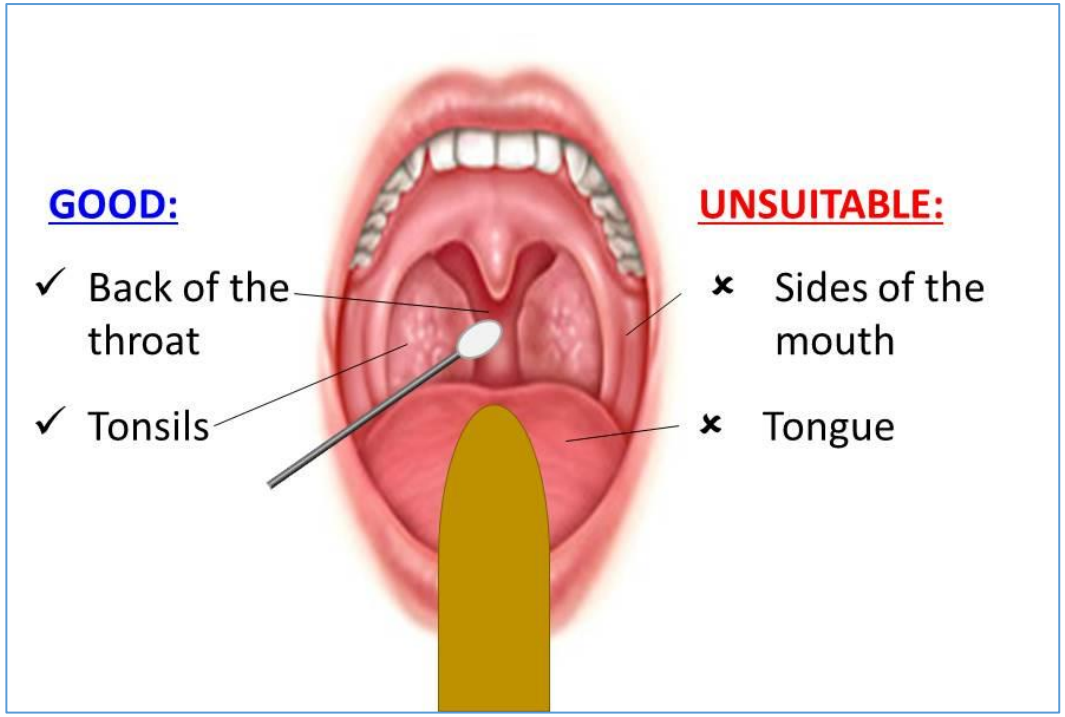
Obtaining material from the nasal cavity

- Specimen from the nasal cavity, is obtained by cotton swab imbedding first into the nasal cavity vertically and then horizontally



Obtaining material from the nasopharynx and throat

- Material is removed from the nasopharynx with a sterile posterior pharyngeal swab, and from the throat with a cotton swab soaked in sterile saline. In this case, the tongue should be fixed with a tounge depressor and the swab should not touch other areas of the oral mucosa.
- In some cases, pharyngeal lavage is performed. To do this, the patient is recommended to rinse with a sterile saline solution.



Principles of the diagnosis of upper respiratory tract infections

- Swabs used to obtain pathological material are delivered to the laboratory in a short time in sterile test tubes.
- Examples as a rule are inoculated on blood agar, chocolate agar, etc., incubated at 37°C for one day, cultured, identified, and tested for antibiotic susceptibility.
- The remaining specimen in the swabs is observed under the microscope after staining by Gram and Neisser methods.
- Virological tests are performed by inoculating the material into cell cultures and chicken embryos.

Microbiology of lower respiratory tract infections

- **Tracheitis and bronchitis -** H. influenzae b serotype, Neisseria, Morachella, streptococci and viruses (ortho- and paramyxoviruses, adenoviruses, coronaviruses). In addition to the causative agents of the acute process, in which the inflammatory process is chronic, there are other bacteria from the genus S. pneumoniae, S. aureus, P. aeruginosa, Klebsiella and Enterobacteriaceae and Candida
- **Pneumonia**
- Primary pneumonia occurs as a result of the entry of pathogenic microorganisms into lung tissue.
- In secondary pneumonia, the pathological process follows any pre-morbid condition called premorbid background (eg, circulatory disorders, immune deficiencies, immunodeficiency, etc.).
- In some cases, pneumonia is not a free disease, but manifests itself as a symptom of any disease. For example, pulmonary tuberculosis, systemic mycoses, ornithosis, Q-fever, legionellosis, etc. accompanied by pneumonia.

Pneumonia - causative microorganisms

- Pneumonia can be caused by a variety of microorganisms - bacteria, mycoplasmas, viruses, fungi and protozoa.
- Among the pathogens are Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Klebsiella pneumoniae, Mycobacterium tuberculosis.
- In relatively rare cases, pneumonia can be caused by enterobacteria, non-spore-forming anaerobes, Candida and other fungi

Pneumonia - causative microorganisms

- ***Bacterial pneumonia*** **-** with Gram-positive bacteria (S. pneumonia, S. pyogenes, S. aureus), Gram-negative bacteria (K. pneumoniae, E. coli).
- ***Viral pneumonia*** develops gradually and has an atypical course and is usually complicated by secondary bacterial pneumonia. Viral pneumonia is mainly caused by RS-virus, adenovirus, influenza and parainfluenza viruses. In relatively rare cases, it is caused by herpesviruses, rhinoviruses, measles, rubella, ECHO-viruses, Coxsackie viruses and coronaviruses.
- ***Atypical pneumonia*** is caused by Mycoplasma pneumoniae, Chlamydia psittaci, Legionella pneumophilia and viruses, most often pneumonia with typical (bacterial) pneumonia.

Principles of diagnosis of lower respiratory tract infections

- **Speciment** may be the sputum, bronchial lavage obtained by bronchoscopy and biopsy of the lungs, lung tissue puncture and aspirate, pleural effusion.
- For microbiological examination, sputum should be taken before the start of antibacterial treatment, or after its reception, after the time necessary for its elimination from the body..
- The morning portion of sputum is taken in a sterile container. Before taking sputum, the patient should rinse his mouth and brush his teeth with boiled water or a weak solution of antiseptics.
- Because bronchoscopy does not contaminate the upper respiratory tract with microflora, the examination of sputum taken by bronchoscopy is more informative.
- If the test is delayed, the sputum can be stored in the refrigerator at 40 ° C for no more than a few hours.

Microbiological examination of sputum

- Various methods are used for microbiological examination of sputum.
- **Microscopic method**. Purulent particles of sputum are identified after washing with an isotonic solution to release microflora of the upper respiratory tract.
- Sputum smears are stained with Gram and, if necessary, Ziehl-Neelsen methods (to detect mycobacteria).
- Microscopy of smears allows to estimate the nature and amount of microflora in sputum, as well as to determine the direction of bacteriological examination.

Microbiological examination of sputum

- It is relatively difficult to determine the etiological role of microorganisms derived from the fact that sputum is contaminated with microbes during passage through the upper respiratory tract and oral cavity.
- Bartlet's score is determined to determine the suitability of sputum for microbiological examination. Bartlett's score is calculated by sputum microscopy. To do this: 1) the number of neutrophils in a field of vision; 2) the presence of muscle fibers; 3) The number of epithelial cells in a field of vision is determined. The high number of neutrophils and muscle fibers is an indicator of the inflammatory process and thus the usefulness of sputum for microbiological examination. The large number of epithelial cells is not an indication of inflammation, but of contamination with saliva.
- A score of 1, 2, or 3 indicates active inflammation, and a score of 0 or lower indicates mild inflammation or contamination with saliva
- neutrophils in one visual field <10 = 0 points; 10-25 = + 1 point; > 25 = +2 points;
- presence of muscle fibers = + 1 point
- number of epithelial cells in a visual field: 10-25 = -1 point; > 25 = -2 points

Microbiological examination of sputum (bacteriological method)

- Purulent particles of sputum are inoculated into a number of nutrient media - blood agar, chocolate agar, differential-diagnostic media, media for anaerobes.
- When fungi are detected in the microscopic preparation, the sputum are inoculated into Saburo or other media for cultivation.
- When tuberculosis or mycoplasma infection is suspected, cultivation is carried out in appropriate media.

Microbiological examination of sputum (bacteriological method)

- Sputum taken with a bacteriological loop is inoculated by spreading it on the surface of a solid nutrient medium.
- 4-sector inoculation is more suitable, which allows to estimate the relative amount of microorganisms in the material and at the same time to obtain a pure culture.
- Samples are incubated for 24 to 48 hours, cultured, identified, and tested for antibiotic susceptiblity

Microbiological examination of sputum (bacteriological method)

- It is relatively difficult to determine the etiological role of microorganisms derived from the fact that sputum is contaminated with microbes during passage through the upper respiratory tract and oral cavity.
- Bacteriological examination of sputum is carried out by **quantitative method** to differentiate the causative microorganism from the microflora of the lower respiratory tract.

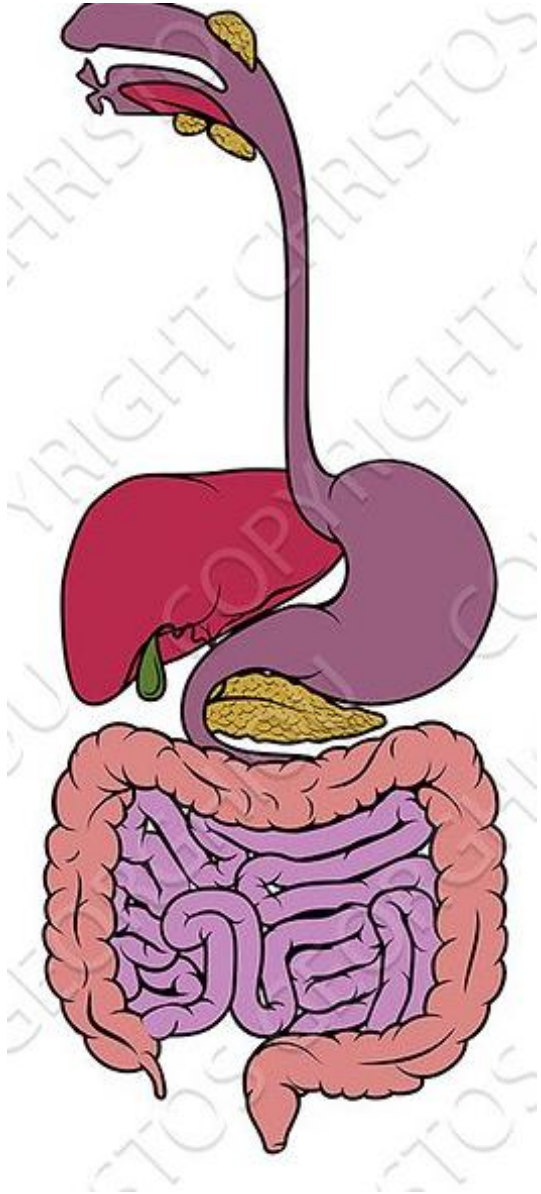
Microbiological examination of sputum (bacteriological method)

- To quantative examination 0.1 ml of carefully homogenized sputum is added in 0.9 ml of isotonic solution to prepare 10 times dilution. Then 0.1 ml of the obtained dilutions is taken and added to another test tube containing 0.9 ml of isotonic solution to obtain 10(2) dilutions.
- After diluting the sputum 10(6)-10(7) times according to this rule, 0.1 ml of the last dilutions is applied to the surface of the blood agar by rubbing it with a spatula. After incubation for 1-2 days at 370C, the results are recorded.
- Microorganisms obtained from dilution 10(6)-10(7) have an etiological role. The growth of microbes in small dilution is estimated as the contamination of sputum with the microflora of the upper respiratory tract.
- It should be considered that the amount of pathogens in the sputum may be low during antibacterial treatment.

Principles of diagnosis of lower respiratory tract infections (serological method)

- The serological method is mainly used in the diagnosis of viral pneumonia.
- Diagnostic indications are a four-fold or greater increase in antibody titers in double-serum samples taken at the beginning of the disease and two weeks later.
- In some infections, IgG and IgM are assigned to the causative agent by ELISA.

Microbiological diagnosis of gastrointestinal tract infections and dysbiosis



Microbiology of oral cavity diseases

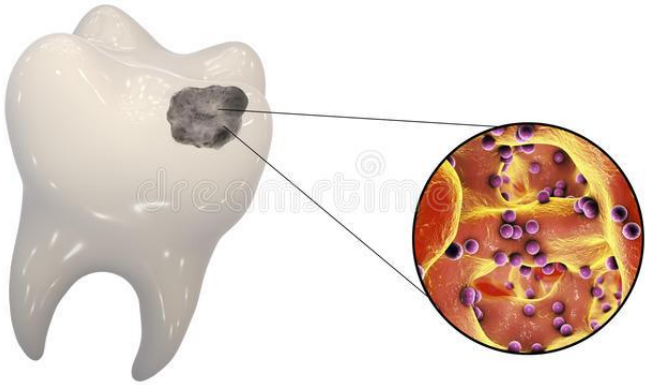
- Diseases of the soft tissues and teeth are distinguished among the diseases of the oral cavity.
- **Stomatitis** is an inflammation of the mucous membrane of the oral cavity. Catarrhal and gangrenous stomatitis are distinguished.
- *Catarrhal stomatitis* is a superficial inflammation of the mucous membrane - staphylococci, neisseria, haemophilus, opportunistic corynebacteria are involved
- *Gangrenous stomatitis* are mainly caused by anaerobes - fusobacteria, bacteroides, peptostreptococci, veilonellas, actinomyses, Vinsent's spirochetes, etc.

Microbiology of oral cavity diseases

- **Gingivitis** is an inflammation of the mucous membranes and tissues of the gums, mainly caused by microorganisms that are part of the gums, including spirochetes, bacteria of the genus Prevotella.
- *Vincent's gingivostomatitis* is characterized by acute hyperemia of the gums and the formation of foci of necrosis by fusobacteria (F.nucleatum), spirochetes (genes T.princelii). Staphylococci, streptococci, peptococci, velonella, actinomycetes, and bacteroids may play a role in the etiology of gingivitis.

Microbiology of oral cavity diseases

- **Caries** - *in the first stage* begins with the formation of spots (plaques) on the surface of tooth enamel. These are mainly gelatinous precipitates of high-molecular carbohydrates - glucans, which are adhesed by acid-forming bacteria. Glucans are mainly secreted by streptococci (S. mutans) (possibly in association with actinomycetes).
- *In the second stage*, streptococci and lactobacilli break down the carbohydrates in these spots to form large amounts of acid (pH <5.0). Such a high concentration of acids leads to demineralization process on an enamel and the formation of caries.



Microbiology of oral cavity diseases

- **Pulpitis** is an inflammation of the dental pulp, which usually occurs after caries as a result of the penetration of microorganisms into the pulp. It consists mainly of lactobacilli, streptococci, bacteroids, peptostreptococci, bacteroids, velonella, proteus and clostridia.
- **Periodontitis**. It occurs as a result of the penetration of microorganisms from the inflamed pulp into the soft and hard tissues surrounding the tooth - the periodontium.
- Microorganisms (streptococci and staphylococci, lactobacilli, fungi, veilonella, bacteroides) produse an enzymes (hyaluronidase, neyraminidase, collagenase) that destroy the connective tissue.

Microbiology of oral cavity diseases

- The entry of microorganisms into the tissues surrounding the teeth can result in periodontal pathology - **periodontitis** and **periodontitis**. Immunopathological processes play an important role in the pathogenesis of these diseases, accompanied by gingivitis and alveolar purulent inflammation.
- Periodontal pathology is an inflammatory-dystrophic process that occurs in the tissues surrounding the teeth. Anaerobes (Porphyromonas, Prevotella, Fusobacterium and Actinobacillus) play an important role in periodontal infections.

The microbiology of gastritis

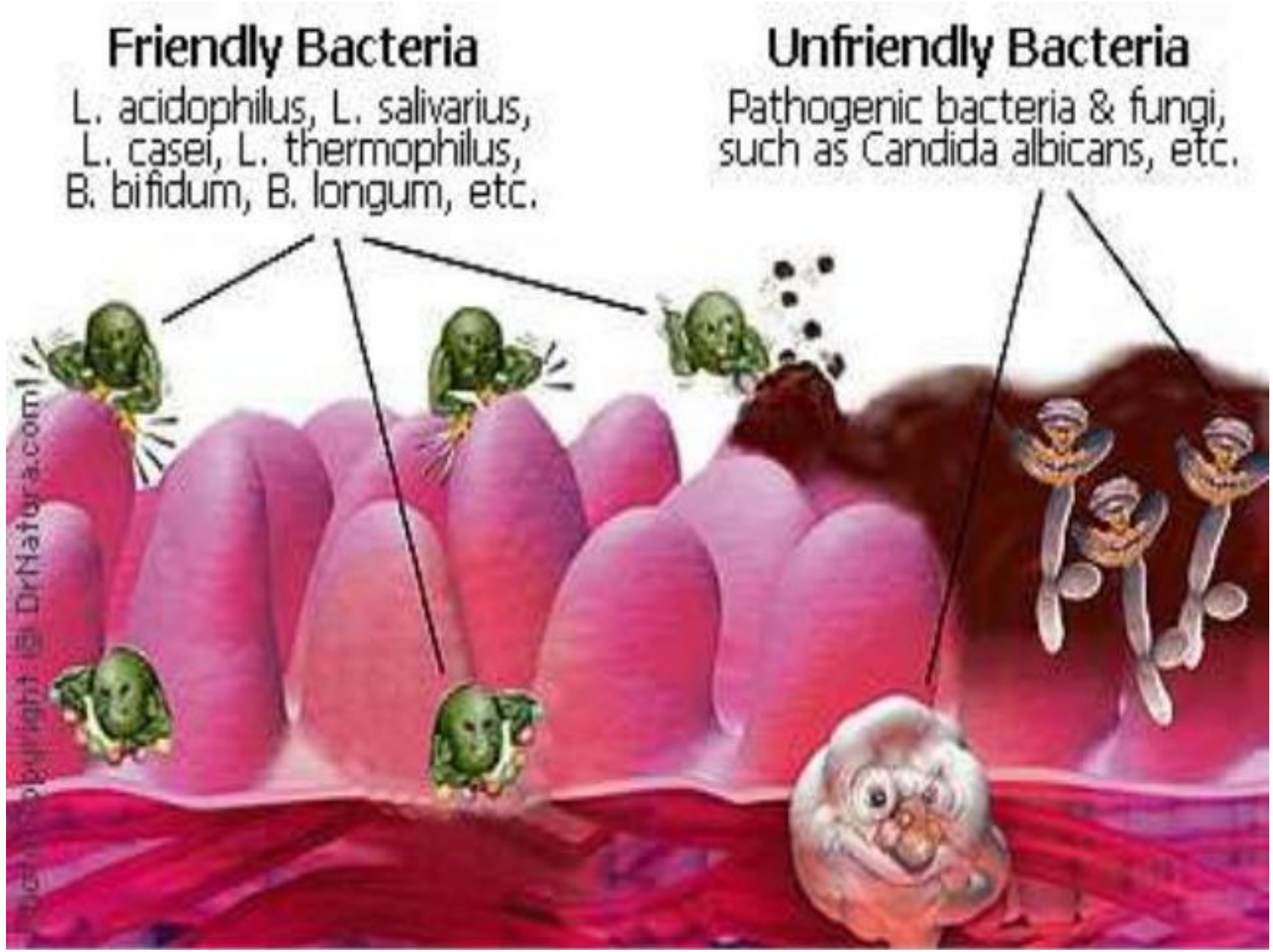
- Inflammation of the gastric mucosa can be caused by various exogenous and endogenous factors. In many cases, gastritis is manifested by inflammatory diseases of the intestine - *gastroenteritis* and *gastroenterocolitis*.
- Salmonella, which are the causative agents of food toxico-infections, play an important role in the etiology of acute gastritis.
- *H.pylori* causes intense inflammation in the mucous membrane of the stomach and duodenum. Acute infection manifests as gastroduodenitis, pain in the epigastric region and is accompanied by nausea. Later, chronic gastritis, gastric and duodenal ulcers may develop. The role of H. pylori in gastric cancer and gastric lymphoma has been confirmed.

Microbiology of acute intestinal infections

- Acute intestinal infections can be caused by bacteria, viruses and protozoa.
- The clinical manifestations of the disease are **enteritis, gastroenteritis, colitis, enterocolitis and gastroenterocolitis**. Diarrhea is one of the main clinical symptoms of acute intestinal infections.
- Bacteria that cause acute intestinal infections - E. coli, S. Typhi, S. Paratyphi A, S. Paratyphi B, Shigella, Vibrio cholerae, Campylobacter jejuni, Yersinia enterocolitica.
- Viruses that cause acute intestinal infections include Norwolk of the Caliciviridae family, as well as Sapporo viruses, adenoviruses, and rotavirus. Invasive protozoa that cause acute intestinal infections.
- Parasites of the genus Entamoeba histolytica, Balantidium coli, Giardia lamblia, Cryptosporidium, Isospora and Sarcocystis, Blastocystis
- The causative agents of food toxicoinfections are Clostridium botulinum, S.aureus, C.perfringens, B.cereus, S.Enteritidis, S.Typhimurium, S.Choleraceus

Dysbiosis and dysbacteriosis

- There is a certain balance between the representatives of the obligate and facultative microflora that make up the normal microflora of the body. This balance is primarily due to the antagonistic effect of the representatives of the obligate microflora on the facultative microflora.
- An imbalance between obligate and facultative microorganisms as a result of various factors leads to dysbiosis and dysbacteriosis.


Friendly Bacteria
L. acidophilus, L. salivarius,
L. casei, L. thermophilus,
B. bifidum, B. longum, etc.
Unfriendly Bacteria
Pathogenic bacteria & fungi,
such as Candida albicans, etc.

Dysbiosis and dysbacteriosis

- Dysbiosis is sometimes classified according to its location (oral cavity, intestines, uterine tract, etc.).
- The term dysbacteriosis is primarily understood as intestinal dysbacteriosis. The development of dysbacteriosis is associated with a reduction in the amount of obligate microflora, which is part of the normal microflora.
- As a result, multiplication of the opportunustic microorganisms - Proteus, Klebsiella, Enterobacter cloaceae, Citrobacter freundii, Serratia marcescens, Hafnia olvei, Morganella morqani, Providenca rettgeri, Pseudomonas aeruginosa, Staphylococcus aureus, Candida species of fungi and so on leds to appropriate diseases. Diseases caused by these microorganisms usually manifest themselves as intestinal infections.
- According to its etiology of dysbiosis, Candida, staphylococcus, proteus, etc. are distinguished.

Principles of microbiological diagnosis of gastrointestinal tract infections

- Stools, vomiting mass, gastric lavage, etc. were used as examination material.
- In some cases, especially in food toxico-infections, food and raw materials that cause the disease are examined.
- The material should be inspected in the first hours after acquisition; otherwise, a material preservative (phosphate-glycerin mixture, etc.) is placed.
- Microbiological examination of feces is carried out by microscopic, bacteriological, parasitological and virological methods.

Principles of microbiological diagnosis of gastrointestinal tract infections

- **Microscopic examination** is carried out by microscopy of native, sometimes Lugol-stained preparations of crushed drip preparations made from feces.
- Microscopic examination is used to assess the condition of digestion, normal microflora, signs of inflammation, as well as the diagnosis of protozoa and helminthiasis.
- The smear prepared from a suspension of faeces in a physiological solution can also be examined after staining by Gram and Sil-Nielsen methods.
- Examination of gram-stained smears reveals the presence of large gram-positive bacilli, such as C. difficile, staphylococci and Candida genus fungi.
- Sil-Nielsen staining reveals acid-fast Cryptosporidium and Isospora protozoa

Principles of microbiological diagnosis of gastrointestinal tract infections

- Bacteriological examination of feces is used for the diagnosis of dysbacteriosis, as well as the detection of bacteria that cause intestinal infections.
- Routine tests are performed by inoculation of a suspension of feces in a saline solution on nutrient medium.
- Fecal samples is performed by 4-sector inoculation with a bacteriological loop on the surface of the solid medium. This method allows to obtain a pure culture, as well as preliminary information about the amount of various microorganisms.

Principles of microbiological diagnosis of gastrointestinal tract infections

- In assessing the etiological role of the obtained cultures, it is important to determine their number, or rather the number of colonies (CFU) formed on the surface of the nutrient medium.
- To do this, it is important to consider the amount of material to be inoculated and the degree of dilutions. The amount of microorganisms is determined per 1 g of feces sample

The following criteria are used in the diagnosis of intestinal dysbiosis and dysbacteriosis:

- Total number of E coli in 1 g of feces sample;
- Relative amount of hemolytic E coli ;
- Presence and relative numbers of opportunistic microorganisms, including Proteus and Candida fungi:
- Total number of bifidobacteria, lactobacilli and bacteroides.

Principles of microbiological diagnosis of gastrointestinal tract infections

- Virological tests are used to detect Norwalk viruses, as well as adenoviruses. Freshly excreted feces or rectal tampons after storage in antibiotic media for 30 min. is inoculated on tissue cultures - the primary culture of the monkey kidney, the culture of human embryo, on the culture of fibroblasts.
- Immune electron microscopy, as well as PCR, is used to detect calciviruses and rotaviruses in feces.