**LESSON 20.
Introduction to clinical microbiology. Healthcare-associated infections. Microbiological diagnosis of infections of the respiratory tract, gastrointestinal tract and microbiota disorders**

**LESSON PLAN:**

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

**Clinical microbiology**

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

**Nosocomial infections**

• Hospital-acquired or nosocomial (from Greek, "nosokomion" - hospital) infections are infections that usually develop 48 hours after admission to the hospital, the source of which is microbes, contaminated medical instruments and equipment, medical personnel, or people visiting patients.

• Hospital-acquired infections are diseases that occur in hospitals, which combine with the main disease and aggravate its course.

• Their causative agents can be both pathogenic and conditional-pathogenic microorganisms.

• In most cases, the causative agents of nosocomial infections are conditionally pathogenic microorganisms.

• The spectrum of the causative agents of nosocomial infections is very wide, they are caused by various viruses, bacteria, fungi and protozoa.

• Staphylococci, enterococci, pneumococci, enterobacteria, P.aeruginosa and other non-fermenting bacteria, anaerobes are more common among the causative bacteria.

• Recently, nosocomial infections caused by respiratory viruses and Candida fungi are increasing.

• Conditionally pathogenic bacteria, which are the causative agents of nosocomial infections, differ according to their following characteristics:

• The causative agents of nosocomial infections are more resistant to antibacterial drugs, antiseptics, disinfectants, physical factors, bacteriophages and bacteriocins.

• Bacteria acquired during nosocomial infections usually have higher virulence.

• Due to the fact that opportunistic microorganisms do not have organotropism, they can cause diseases in any organs and seeds of the organism through various mechanisms.

• Heterogeneity (antigen variability, etc.) in the population of the causative agents of nosocomial infections is higher in comparison with other microorganisms.

• Examination materials are selected depending on the localization and character of the disease.

• The microscopic method allows to draw an approximate conclusion about the character of the causative agent and to determine the direction of cultural methods. Depending on the nature of the examination material, the prepared smears are stained with appropriate methods and then subjected to microscopy.

• The cultural method is the main method of microbiological diagnostics in nosocomial infections and is based on the cultivation of pathological materials in nutrient media, the acquisition and identification of a pure culture of the causative agent. It is important to determine the sensitivity of the culture to antibiotics and other antimicrobial chemical therapeutics.

**Upper respiratory tract (anatomical information and normal microflora)**

• Inflammatory diseases of the upper respiratory tract - nasal cavity, pharynx (nasopharynx, oral part of the pharynx) and larynx are often caused by conditionally pathogenic microorganisms.

• More non-pathogenic corynebacteria, coagulase-negative staphylococci, alpha-hemolytic streptococci, neisseria, and sometimes potentially pathogenic bacteria in the mucous membrane of the nasal cavity. Bacteria - S.aureus, beta-hemolytic streptococci, S.pneumoniae, E.coli, Proteus is revealed.

• Rhinitis and sinusitis (hemorrhitis, ethmoiditis, etc.) - adenoviruses, rhinoviruses, coronaviruses, etc.

• Pharyngitis, or angina (inflammation of the pharyngeal arches, soft palate and oral part of the pharynx) and tonsillitis (inflammation of the tonsils).

• Catarrhal pharyngitis – ortho- and paramyxovirus, adenovirus, coronavirus, herpes simplex virus and Coxsackie virus

• Purulent pharyngitis - in approximately 90% of cases, the causative agent is S.pyogenes, in the remaining cases, other bacteria, especially S.aureus, S.pneumoniae, C.diphtheriae, B.pertussis, H.influenzae, etc.

• Nasopharyngitis - N. meningitidis, other bacteria from the genus Neisseria

• Laryngitis - parainfluenza virus, C. diphtheriae, etc.

• Materials for microbiological diagnosis of upper respiratory tract diseases are mainly obtained with sterile swabs.

• The material from the nasal cavity is obtained by inserting a cotton swab into the nasal cavity first vertically and then horizontally.

• The material is taken from the nasopharynx with a sterile back-swallow swab, and from the larynx with a cotton swab soaked in sterile physiological solution. At this time, the tongue should be fixed with a spatula and the tampon should not touch other areas of the oral mucosa.

• In some cases, swallowing water is examined. For this, the patient is suggested to gargle with sterile physiological solution.

• Swabs used to obtain pathological materials are delivered to the laboratory in a short time in sterile test bottles.

• Samples are blood white, chocolate white, etc. as appropriate. is inoculated, incubated for one day at 370C temperature, pure culture is obtained, identified and its sensitivity to antibiotics is studied.

• Smears prepared from the material remaining in the buffer are subjected to microscopy after being stained by Gram and Neisser methods.

• Virological tests are carried out by inoculating materials into cell cultures and chicken embryos.

**Microbiology of lower respiratory tract infections**

• Tracheitis and bronchitis - H.influenzae b serotype, Neisseria, Moraxella, streptococci and viruses (ortho- and paramyxoviruses, adenoviruses, coronaviruses, etc.). In addition to the causative agents of the acute process, in which the inflammatory process becomes chronic, S. pneumoniae, S. aureus, P. aeruginosa, Klebsiella and other bacteria from the family Enterobacteriaceae, fungi of the genus Candida.

• Pneumonia

• Primary pneumonias occur as a result of the penetration of lung tissue by micro-organisms.

• In the case of secondary pneumonia, the pathological process develops after any premorbid condition called premorbid background (for example, blood circulation disorders, immune deficiencies, aspiration of vomit mass, etc.).

• In some cases, pneumonia is not an independent disease, but appears as a symptom of any disease. For example, pulmonary tuberculosis, systemic mycoses, ornithosis, Q-fever, legionellosis, etc. It is accompanied by pneumonia.

• Pneumonia can be caused by various microorganisms - bacteria, mycoplasmas, viruses, fungi and protozoa.

• Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Klebsiella pneumoniae, Mycobacterium tuberculosis are more common among the causative agents.

• In relatively few cases, pneumonia can be caused by enterobacteria, non-spore forming anaerobes, Candida and other fungi.

• Pneumonias of bacterial origin - with Gram-positive bacteria (S.pneumonia, S.pyogenes, S.aureus), with Gram-negative bacteria (K.pneumoniae, E.coli, H.influenzae).

• Viral pneumonias begin gradually and have an atypical course and are usually complicated by secondary bacterial pneumonias. Viral pneumonias are mainly caused by RS-virus, adenovirus, influenza and parainfluenza viruses. In relatively few cases, it is caused by herpesviruses, rhinoviruses, measles, mumps, ECHO-viruses, Coxsackieviruses and coronaviruses.

• Atypical pneumonias - caused by Mycoplasma pneumoniae, Chlamydia psittaci, Legionella pneumophilia and viruses, in most cases they have a mild course compared to typical (bacterial) pneumonias and are difficult to treat. differs by having

• Examination material can be sputum, bronchial contents obtained by bronchoscopy, trachea obtained by puncture and lung biopsy, lung tissue punctate and aspirate, pleural fluid.

• It is necessary to collect sputum for microbiological examination before the start of antibacterial treatment or after the time necessary for its elimination from the body after its reception.

• The morning portion of sputum is taken in a sterile container. Before collecting sputum, the patient should rinse his mouth with boiled water or a weak solution of antiseptics, and brush his teeth.

• Examination of sputum taken by bronchoscopy is more informative, since it is not contaminated with mycoflora of the upper respiratory tract.

• If the examination is delayed, the sputum can be kept in the refrigerator at 40C for not more than a few hours.

• Various methods are used for microbiological examination of sputum.

• Microscopic method. Purulent particles of sputum are examined after washing with an isotonic solution to free them from the microflora of the upper respiratory tract.

• Smears prepared from sputum are stained with Gram and, if necessary, Sil-Nelsen methods (to detect mycobacteria).

• Microscopy of smears allows to make an approximate judgment about the nature and amount of microflora in sputum, as well as to determine the direction of bacteriological examination.

• It is relatively difficult to determine the etiological role of the acquired microorganisms, since sputum is contaminated with microbes while passing through the upper respiratory tract and oral cavity.

• Bartlett's score is used to determine the suitability of sputum for microbiological examination. Bartlett score is calculated as a result of sputum microscopy. For this: 1) the number of neutrophils in one field of vision; 2) presence of muscle fibers; 3) the number of epithelial cells in one field of vision is determined. A high number of neutrophils and muscle fibers is an indicator of the inflammatory process and thus the suitability of sputum for microbiological examination. A high number of epithelial cells is not an indication of inflammation, but of saliva contamination.

• Scores of 1, 2, or 3 indicate active inflammation, scores of 0 or lower indicate mild inflammation or salivary contamination

• Neutrophils <10 in one visual field =0 points; 10-25 =+1 point; >25 = +2 points;

• presence of muscle fibers = +1 point

• The number of epithelial cells in one visual field: 10-25 = -1 point; >25 = -2 points

• Purulent particles of sputum are inoculated into a series of nutrient media - blood agar, chocolate agar, differential-diagnostic media, media for anaerobes.

• When fungi are detected in the microscopic preparation, they are inoculated into Saburo or other media to cultivate the fungi.

• If tuberculosis or mycoplasma infection is suspected, culture is performed in appropriate media.

• Sputum taken with a bacteriological loop is inoculated by spreading it on the surface of a solid nutrient medium.

• 4-sector inoculation is more convenient, which allows to estimate the relative amount of microorganisms in the material and to obtain a pure culture at the same time.

• Samples are incubated for 24-48 hours, pure culture is obtained, identification and sensitivity to antibiotics is determined.

• It is relatively difficult to determine the etiological role of the acquired microorganisms, since sputum is contaminated with microbes while passing through the upper respiratory tract and oral cavity. In order to differentiate the causative microorganism from the microflora of the lower respiratory tract, bacteriological examination of sputum is carried out using a quantitative method.

• To quantitatively examine sputum, carefully homogenize the material, prepare 10 times rinsing by adding 0.9 ml of isotonic solution to 0.1 ml of homogenized sputum. Then, 0.1 ml of the received rinses is taken and added to another test bottle containing 0.9 ml of isotonic solution and its 102 rinses are obtained.

• According to this rule, after rinsing the sputum 106-107 times, 0.1 ml of the last rinses are smeared on the surface of the bloody agar with a spatula. After 1-2 days of incubation at 370C, the results are recorded.

• Microorganisms obtained from 106-107 rinses have an etiological role. Microbial development in low rinses is estimated as contamination of sputum with microflora of the upper respiratory tract.

• It is necessary to take into account that the amount of causative agents in sputum may be low when carrying out antibacterial treatment.

• The serological method is mainly used in the diagnosis of viral pneumonias.

• A 4-fold or more increase in the titer of antibodies in duplicate blood serum samples taken at the beginning of the disease and two weeks later is a diagnostic indicator.

• In some infections, IgG and IgM against the causative agent are determined by IFA.

**Microbiology of diseases of the oral cavity**

• Among the diseases of the oral cavity, diseases of its soft tissue and teeth are distinguished.

• Stomatitis is an inflammation of the mucous membrane of the oral cavity. Catarrhal and ulcerative-gangrenous stomatitis are distinguished. Catarrhal stomatitis is a superficial inflammation of the mucous membrane, its occurrence is caused by staphylococci, neisseria, hemophilic bacteria, conditional-pathogenic corynebacteria, etiol of ulcerative-gangrenous stomatitis. and anaerobes - fusobacteria, bacteroids, peptostreptococci, velonella, actinomycetes and Vincent's spirochetes prevail in ogia.

• Gingivitis is an inflammation of the mucous membrane and seed of the gums, mainly caused by microorganisms included in the composition of dental plaque, including spirochetes, bacteria of the genus Prevotella. Vincent's gingivostomatitis, characterized by acute hyperemia of the gums and the formation of necrotic foci, is caused by fusobacteria (F. nucleatum), spirochetes (T. vinsantii), as well as bacteria of the genus Prevotella. Staphylococci, streptococci, peptococci, velonella, actinomycetes, bacteroids can play a certain role in the etiology of gingivitis.

• Caries - in the first stage, it begins with the formation of spots (plaques) on the surface of the tooth enamel (enamel layer). They mainly consist of a gelatin-like precipitate of high-molecular carbohydrates - glucans, to which acid-forming bacteria adhere. Glucans are mainly secreted by streptococci (S. mutans) (possibly in association with actinomycetes).

• In the second stage, streptococci and lactobacteria produce a large amount of acid (pH<5.0) by decomposing the carbohydrates found in these spots. Such a high concentration of acids causes enamel demineralization and caries formation.

• Pulpitis is an inflammation of the dental pulp, which usually occurs as a result of the penetration of microorganisms into the pulp after caries. More lactobacteria, streptococci, bacteroides, peptostreptococci, bacteroides, velonellas, proteasomes and clostridia are involved in the association.

• Periodontitis. It occurs as a result of the penetration of microorganisms from the inflamed pulp into the soft and hard seeds covering the tooth - the periodontium. As a rule, the microorganisms (streptococci and staphylococci, lactobacteria, corynebacteria, fungi, velonella, bacteroids) that are in association are separated from the connecting seed. synthesizes enzymes (hyaluronidase, neuraminidase, collagenase) that break down their separate components and deepens the inflammatory process.

• The inclusion of microorganisms in the seeds covering the teeth can result in the pathology of the periodontium - periodontitis and periodontosis. Immunopathological processes are important in the pathogenesis of these diseases accompanied by gingivitis and alveolar purulent inflammation.

• Periodontal pathologies are accompanied by the presence of inflammatory-dystrophic processes occurring in the seeds covering the teeth, the breakdown of collagen, the absorption of alveolar protrusions, bone seeds, and the loss of teeth. strike Anaerobes (Porphyromonas, Prevotella, Fusobacterium and Actinobacillus) are important in periodontal infections.

**Microbiology of gastritis**

• Inflammation of the mucous membrane of the stomach can be caused by various exogenous and endogenous factors.

• In many cases, gastritis is manifested by inflammatory diseases of the intestines - such as gastroenteritis and gastroenterocolitis. In the etiology of acute gastritis, Salmonella bacteria, which are the causative agents of food poisoning, are of certain importance.

• H.pylori causes intense inflammation in the mucous membrane of the stomach and duodenum by disrupting the integrity of the epithelial membrane. Acute infection manifests as gastroduodenitis, accompanied by epigastric pain and 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.

• 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, Vibrio parahaemolyticus and Plesiomonas shi. gеllоides, C.difficile

• Viruses that cause acute intestinal infections are Norwalk from the Caliciviridae family, as well as Sapporo viruses, Adenoviruses, Rotavirus.

• Invasive protozoa causing acute intestinal infections. Parasites from the genus Entamoeba histolytica, Balantidium coli, Giardia lamblia, Cryptosporidium, Isospora and Sarcocystis, Blastocystis

• The causative agents of food poisoning are Clostridium botulinum, S. aureus, C. perfringens, B. cereus, S. Enteritidis, S. Typhimurium, S. Choleraesuis

• There is a certain balance between the representatives of the obligate and facultative microflora that make up the normal microflora of the organism. First of all, this balance is related to the antagonistic effect of obligate microflora representatives on facultative microflora.

• Disturbance of this balance between obligate and facultative microorganisms as a result of the influence of various factors leads to the occurrence of a condition called dysbiosis and dysbacteriosis.

• Sometimes dysbioses are classified according to their localization (oral cavity, intestines, childhood tract, etc.).

• The term dysbacteriosis is primarily understood as intestinal dysbacteriosis. The development of dysbacteriosis is related to the decrease in the amount of obligate microflora, which is part of the normal microflora. As a result, conditionally pathogenic microorganisms in the facultative microflora - Proteus, Klebsiella, Enterobacter cloaceae, Citrobacter freundii, Serratia marcescens, Hafnia olvei, Morganella morgani, Pro vidеncа rеttgеri, Pseudomonas aeruginosa, Staphylococcus аureus, Candida fungi, etc. corresponding diseases caused by its proliferation occur. Diseases caused by these microorganisms usually manifest as intestinal infections.

• According to its etiology, Candida, staphylococcus, protein, etc. origin dysbioses are distinguished.

• Due to self-regulation mechanisms, the microflora composition is quite stable. Therefore, it is necessary to distinguish real dysbacteriosis and dysbiosis from temporary dysbacterial and dysbiotic reactions. In the latter cases, changes in the normal microflora are temporary, short-term and do not require correction.

• In real dysbiosis and dysbacteriosis, changes in the composition and function of normal microflora are long-term, with various disorders - diarrhea, constipation, colitis, malignant tumors, allergies, hypovitaminosis, hypo- and hypercholesterolemia, hypo- and hypertension, caries, arthritis, various pathologies of the liver, etc. . accompanied by

• Widespread and uncontrolled use of antimicrobial drugs plays a major role.

• In addition, other factors - concomitant diseases, especially intestinal infections, helminth and parasite infestations, hormonal and chemical therapy, stress, etc. factors also play a role.

• The modern era, when environmental conditions are getting more and more intense, is accompanied by a wide spread of dysbacteriosis.

• The development of dysbacteriosis is related to the decrease in the amount of obligate microflora, which is part of the normal microflora.

• As a result, conditionally pathogenic microorganisms in the composition of facultative microflora - staphylococci, Proteus, Pseudomonas, etc. relevant diseases occur due to the growth of bacteria of the genus Candida, as well as fungi of the genus Candida.

• According to its etiology, fungi, staphylococcus, protein, etc. origin dysbioses are distinguished.

• Sometimes they classify dysbioses according to their localization (oral cavity, intestines, uterus, etc.).

• Long-term changes in the composition and function of the normal microflora cause symptoms accompanying various disorders.

• Among them are diarrhea, constipation, colitis, malignant tumors, allergy, hypovitaminosis, hypo- and hypercholesterolemia, hypo- and hypertension, caries, arthritis, various pathologies of the liver, etc. more common.

• Feces, vomit mass, gastric lavage, etc. as examination material. is used.

• In some cases, food products and raw materials that cause illness are examined, especially in cases of food poisoning.

• The material should be examined in the first hours after its acquisition; otherwise, the material is placed in a preservative (phosphate-glycerin mixture, etc.).

• Microbiological examination of feces is carried out by microscopic, bacteriological, parasitological and virological methods.

• Microscopic examination is carried out by microscopy of native, sometimes Lugol's stained preparations of crushed drop preparations prepared from feces.

• Microscopic examination is used for evaluation of digestion, normal microflora condition, signs of inflammation, as well as diagnosis of protozoa and helminthosis.

• Smears prepared from the suspension of feces in a physiological solution can be subjected to microscopy after being stained by Gram and Sil-Nielsen methods.

• Gram-stained smears can reveal large gram-positive bacteria, C. difficile, staphylococci, and Candida fungi.

• Acid-resistant Cryptosporidium and Isospora protozoa can be detected by Sil-Nilsen staining.

• Bacteriological examination of feces is used for the diagnosis of dysbacteriosis, in addition to detecting bacteria that cause intestinal infections.

• Routine examinations are carried out by inoculation of a suspension of faeces in a physiological solution into nutrient media.

• Differential-diagnostic media for obtaining enterobacteria - Endo (or McConkey's medium), Levin (or eosin-methylene brother medium), bismuth-sulfite agar for salmonella, blood for staphylococci, eggs, etc. honey-salt agar, for pseudomonads and bacilli Meat-peptone agar, Saburo medium is used for mushrooms.

• Feces are inoculated onto the surface of the solid food medium by the 4-sector inoculation method with a bacteriological loop. This method allows to obtain a pure culture, as well as to obtain preliminary information about the amount of various microorganisms.

• Determination of their number, or rather the number of colonies formed on the surface of the nutrient medium, is of great importance in assessing the etiological role of the obtained cultures.

• For this, it is important to consider the amount of inoculated material and the degree of rinsing. The amount of microorganisms is calculated according to the amount of 1 g of feces.

• Total number of intestinal bacteria in 1 g of stool sample;

• Relative amount of hemolytic intestinal bacteria;

• Presence of opportunistic bacteria, including Proteus bacteria and Candida fungi, and their relative amount:

• Amount of bifidobacteria, lactobacteria and bacteroides.

• Virological tests are used to detect Norwalk viruses as well as adenoviruses. Freshly secreted feces or rectal tampons in antibiotic environments for 30 minutes. after storage, it is inoculated into seed cultures - monkey kidney primary culture, human embryo diploid fibroblast culture.

• Immune electron microscopy and PCR are used to detect caliciviruses and rotaviruses in feces.

• Serological examinations. In intestinal infections caused by Norwalk viruses and rotaviruses, during the acute and convalescent periods of the disease, a four-fold or more increase in the titer of anti-virus antibodies in the blood sample by IFA confirms the diagnosis.

• First of all, it is done by identifying and eliminating the factors that cause it.

• Removal of conditionally pathogenic microflora (selective decontamination) that develops against this background is one of the important conditions.

• Probiotics (eubiotics) are used to restore the microflora.

• As eubiotics, mainly obligate representatives of normal intestinal microflora - bifidobacteria, lactobacteria, intestinal bacteria, enterococci, etc. bacteria are used.

• For this purpose, bacterial preparations are applied in the form of lyophilized dry powder, tablets, as well as extracts.