Guidelines ratified on meeting of the Central methodical committee of Zaporizhzhya state medical university (protocol numbers 4 (26.02.2015) and it is recommended for the use in educational process for foreign students.

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The independent practical work of students is an important part of the syllabus in the course of microbiology, virology, immunology. It helps students to study this fundamental subject.

The systematic independent work enables to reach the final goal in the students’ education. It is also important while preparing the students for their future clinic work with patients. These theoretical material, questions and tests help students to get ready for examination.

The methodical manual for practical lessons on microbiology, virology, immunology for the medical students of 2-3 year of the study are approved by the Central Methods Board of ZSMU as a methodical manual on practical lessons for students of the medical faculty.
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Clinical microbiology:

Definition of the term:
- It is a division of medical microbiology which includes the study of:
  - infectious diseases which occur in hospitals where the patients are getting treatment in the case of non-infectious diseases,
  - these infections named hospital-acquired (iatrogenic or nosocomial) ones.

Clinical microbiology: main subjects
The main subjects are:
- study of hospital-acquired infections:
  - their aetiology
  - pathogenesis
  - specificity of the immune response
- development and applying of the methods which include:
  - laboratory diagnosis
  - specific treatment
  - prophylaxis

Clinical microbiology: methods
- The methods which are used by clinical microbiology includes usual methods characteristic for medical microbiology but the main method is:
  - the method of growing of bacterial culture and the quantitative aspect of the method (the number of the bacterial cells in the specimens) is especially important

Clinical microbiology: objects of the study
- common opportunistic pathogens
hospital environment.

In hospital hospitals millions patients annually arrive.

The main task of collectives of these establishments is fast recovery of health of the hospitalised patients and creation of safe conditions of their stay in these hospitals in respect of possible infection with an hospital infection.

In the scientific plan the given problem dares joint efforts of clinical physicians, epidemiologists, microbiologists.

Specific microbiological problems in somatic hospitals exist for a long time, but the concept about clinical microbiology as independent section of the medical microbiology differing from infectious or sanitary, has started to develop several decades ago and continues to be improved now.

Formation of the given section is connected, mainly, with constant evolution of bacteria and the illnesses caused by them which rates of development have sharply increased in second half XX centuries.

For this time the quantity of infectious diseases in not infectious clinics of a therapeutic and surgical profile has considerably increased.

Principal cause of the created situation is distribution of is conditional-pathogenic microorganisms as a result of wide, sometimes irrational application of chemotherapeutic preparations, especially antibiotics, introductions in medical practice of the procedures conducting to infringement of integrity of external covers (a skin and mucous membranes), etc.

It has led to wide circulation polyresistant to antibiotics and hospital ecological variations of bacteria, to increase of weight of the hospital, chronic, mixed infections and a sepsis.

According to it clinical microbiology define as the section of medical microbiology investigating microbiological aspects of an aetiology, patogntntsis and immunity of microbical diseases in not infectious clinic developing and
realising methods of their laboratory diagnostics, specific therapy and preventive maintenance.

Specificity of clinical microbiology consists that it investigates one group of microbes - is conditional-pathogenic, one group of diseases - opportunistic infections, and one anthropogenous ecological system - hospitals.

Proceeding from it, problems of clinical microbiology consist in the following:

1) Research of biology and role of is conditional-pathogenic microorganisms in an aetiology and патогенезе infectious diseases of the person, and also in maintenance of its health as they are normal inhabitants of an organism;

2) Working out and use of methods of the microbiological diagnostics, specific therapy and preventive maintenance of the microbic diseases meeting in not infectious medical institutions;

3) Research of microbiological aspects of problems of hospital infections, dysbacteriosis, medicinal stability of microbes;

4) Microbiological substantiation and the control over antimicrobial actions in treatment-and-prophylactic establishments.

CONDITIONAL-PATHOGENIC MICROORGANISMS

It is the big and diverse group in the regular relation, causing in the person of illness under certain conditions.

Is conditional-pathogenic microorganisms meet among bacteria, mycoplasmas, fungi, the elementary. Some viruses, for example, an alpha-gerpesvirusy of 1 and 2 types, beta-gerpesvirus to many signs are close to them.

In a modern pathology of the person it is supposed aetiological a role about 100 kinds of is conditional-pathogenic microorganisms.

Among them representatives of following sorts have major importance:
Staphylococcus, Streptococcus, Peptostreptococcus, Escherichia, Enterobacter, Klebsiella, Citrobacter, Serratia, Proteus, Pseudomonas, Haemophilus, Acinetobacter, Bacteroides, Bacillus, Mycobacterium, Mycoplasma, Candida, Cryptococcus, Pneumocysta.

In the ecological relation is conditional-pathogenic microorganisms are non-uniform. Among them there is a group of living the kinds which inhabitancy is foodstuff, soil, water, an organic waste of activity of people, medical products.

The majority of these kinds are capable to live in different biotops of a human body and under certain conditions to cause diseases.

However for kind continuation the live environment for them is not obligatory.

In hospital hospitals from the given group of microbes meet legionells, sarcines, eprotes, klepsiella of a pneumonia.

For example, salmonellas, also it is possible to carry some kinds of parasites of animals to is conditional-pathogenic bacteria though many of them are parasites of the person. The listed bacteria can be with it in symbiotic relations.

However under certain conditions they enter competitive relations and cause infectious diseases though it and does not give them biological advantages, moreover, can lead to loss of the owner.

Pathogenicity. Development of infectious process, first of all, depends on entrance gate of an infection and ability of the activator to adapt in them.

Autohtoms for defined biotops is conditional-pathogenic kinds are well adapted for the owner and easily adapt at hit in similar biotops other person.

Efficiency of adaptation аллохтонных is conditional-pathogenic microorganisms appreciably depends on coincidence of an ecological niche with autohtoms kinds and character of ecological communications between them: coincidence of an ecological
niche at competitive relations interferes with adaptation in a place of entrance gate and on the contrary.

The same law is characteristic for action элиминирующих mechanisms of entrance gate of an infection of the owner: they are active against obligatno-pathogenic kinds, are less active or are at all inactive against is conditional-pathogenic kinds. The exception is made by blood and a lymph which action is directed as against obligat - and is conditional - pathogenic microbes, and the first more resistant to it.

In the adaptation mechanism проникших in an organism of bacteria crucial importance have ligando-retseptornyye mutual relations between bacteria and the cellular receptors, coming to an end with adhesion.

The surface of epithelial cages of healthy people almost is entirely occupied by representatives microflora.

Moreover, epithelium with the layer which has stuck to it of bacteria is the uniform structure which is carrying out protective function. It testifies from volume, that in infection development essential value have not adhesion properties of conditionally - and obligat-pathogenic bacteria, and their ability to overcome competing action microflora.

Invasion in depth of fabrics and aggression - suppression phagocytosis and immunocompetent cages of an organism of the owner are absent or are found out only at separate штаммов or sick-lists ecological variations.

Therefore development of opportunistic infections occurs or at passive drift of the activator in immunodeficient an organism, or at considerable quantity receipt endotoxin in blood in case of mass destruction gram negative bacteria.

As is conditional-pathogenic bacteria on produce albuminous exotoxins and are not capable to endocellular, they exotoxin can be considered as the universal factor of pathogenicity. A target for action эндотоксина are cages of many bodies of the person that defines identity or affinity of the defeats caused by it.
As toxicity эндотоксина is insignificant, only its high concentration can cause clinical defeats.

Is conditional-pathogenic bacteria produce a considerable quantity various of exoferments: gialuronidase, coagulase, fibrinolisine, neuromenidase, etc., rendering action on molecules free or a part of cages. The listed factors of virulence, except endotoxin, come to light at is conditional-pathogenic bacteria in the incomplete and different complete set.

**Populations.** For various kinds of is conditional-pathogenic bacteria - inhabitants of a human body (staphilococci, pseudomonads, enterobacteria, etc.) - are characteristic large number of individuals, heterogeneity and variability of populations, and also participation in various of microbiocenosis.

**Heterogeneity of populations** of is conditional-pathogenic bacteria is shown almost to all signs, but especially it is expressed in antibioticoresistent, stability to antiseptics, bacteriophages and bacteriocines. Well-known high heterogeneity of antigen structure of the majority of is conditional-pathogenic bacteria which creates the big complexities in identification of the allocated cultures.

The principal cause of heterogeneity of populations of is conditional-pathogenic bacteria in the pathological centres consists that infecting of the person occurs a heterogeneous pool of the activator, and in the course of illness occurs superinfecting by ecological variations.

Populations of is conditional-pathogenic bacteria not only geterogenuous, but also change in the course of treatment towards transition from sensitive variants to polyresistant, from nonhospital ecological variations to sick-lists.

Elamination of separate ecological variations from population it is supervised by immune system of an organism, antimicrobial and immunostimulation preparations.

Considering heterogeneity and variability of populations of the is conditional-pathogenic bacteria causing opportunistic infections, it is practically important:
To investigate the big number of cultures of one kind in the course of microbiological diagnostics;

At a choice of chemotherapeutic means to be guided by variants and штаммы the activator, resistance possessing the highest level to antibiotics and antiseptics;

To observe in a course of disease for quantitative and qualitative changes in structure of population of the activator and to correct the treatment scheme;

To warn a superinfection as by isolation of the pathological centres, and sharp decrease in mass character microbic dissimilation of objects of an environment.

**Microbiosinosis.**

Is conditional-pathogenic microbes live in a kind of communities - microbiosinosis, including many populations of different species of microorganisms. In the regular relation in structure microbiosinosis healthy people representatives different taksons of bacteria, fungi, the elementary contain, viruses, in ecological - in it enter kinds which can be freely living, not pathogenic, is conditional - also obligat - pathogenic microorganisms.

Members microbiosinosis occupy in biotop certain ecological niches and are among themselves in certain parities: symbiosis, a competition. These factors basically define quantitative parities between them.

Microbiosinosis of the people who are in hospital hospitals, differ from those out of a hospital, first of all colonization of biotops sick-lists ecological variations.

Frequency of colonisations above at immunodefishence persons, newborns and the patients who are long time on hospitalisation. It is high and at the medical workers working in given establishments.

These differences concern also ability decrease to autostabilisation, strengthening of competitive mutual relations between fellow members microbiosinosis and its separate representatives with an organism of the owner, and also in the increased frequency intrapopulation an exchange.
Specific and alternative structure microbiosinosis define by a quantity establishment CU (colony units) in 1 ml of an investigated material. For etymologically significant number CU /ML accept for bacteria 105, for fungi and the elementary - 103 - 104 and more. In case of allocation from a pathological material of several variants or species of microorganisms for the leader of the activator assume a dominating air or a variant.

Auxiliary value in an establishment etiology roles of the allocated culture has repeated allocation (in a day or earlier) the same kind or biovar, detection at it the same factors of virulence, increase in whey of sick antibodies to the given culture in 4 times and a number of other tests.

**OPPORTUNISTIC INFECTIONS**

**Opportunistic microorganisms: the main groups**

1. Gram-positive cocci (staphylococci, pneumococci)
2. Gram-negative enteric rods
3. Pseudomonades
4. Fungi Candida
5. Fungi Pneumocystis

**Opportunistic microorganisms : main characteristics**

1. Ecological grouping
   - Free-living
   - The patient’s indigenous micro-flora
2. Conditions for the realisation of the pathogenicity by opportunistic microorganisms:
   - high adaptive capacities in microbial population
   - endotoxin
• producing of toxic enzymes

3. Characteristics of the opportunistic population:
   • heterogeneity
   • changeability

4. Specificity of the methods applied for the diagnosis of nosocomial infections:
   • it is necessary to analyse many different strains
   • it is necessary to pay special attention to high resistant strains (bacteria resistant to drugs, antiseptics and disinfectants)

**Opportunistic infection**

**Definition of the term:**
- The infections caused by opportunistic pathogens (more frequently by their associations).

**Opportunistic infections: conditions for their development**
1. High inoculum of pathogens.
2. Debilitation of hospitalised patients.
3. Infection by more virulent pathogens.

**Opportunistic infections: specific features**
- Tropism to many organs
- Clinical symptoms usually dependent on the site of localisation of the infectious process: symptomatology may be vague or atypical
- Opportunistic diseases often are getting:
  - chronic
  - generalised
  - could result in septicopyemia
- Problems for the therapy:
drug resistance of wide spectrum
- low resistance of human organism to the infection
- The infections may be endogenous
- The hospital – acquired infections are dominant among the opportunistic ones.

**Opportunistic infections : laboratory diagnosis**

Rapid diagnosis of opportunistic infections is important to a favourable prognosis for the patient.

Laboratory diagnosis includes:
- Aetiology - isolation of the opportunistic pathogen especially when the pathogen which has been isolated:
  - is occurring in high titre,
  - demonstrates high virulence,
  - it has been isolated from unusual site in human organism.
- The status of the immune response of the patient should be taken into consideration.
- Epidemiological aspects should be considered such as:
  - the source of the infection
  - factors of the transmission of the infection

Development and current of opportunistic infections (an armour. *opportunas* - inclined to disease) are defined by three groups of factors: properties of the activator, a macroorganism and environment condition.

From the activator in infection occurrence the infecting dose of the activator and presence at it a certain set of factors вирулентности has crucial importance high and heterogeneous on pathogenicity.

From a human body - infringement of integrity of covers and, that is the most essential, immunodefishence conditions.
Value of environment is connected with presence of factors of transfer of the activator from the infected person not infected.

Activators of opportunistic infections have no strictly expressed tropism owing to what the same kind can cause various nosological forms (a bronchitis, a meningitis, a pyelonephritis, etc.). In turn same nosological the disease form (the pneumonia, an osteomyelitis, a sepsis, etc.) can be caused any is conditional pathogenic microorganism.

Opportunistic infections are often caused by association of microorganisms. Mixed, or mix-infections, result simultaneous, and more often consecutive infection of the person with several kinds of activators.

The clinical picture of opportunistic infections is a little specific. It depends in большей to a measure on defeat localisation, than from an activator kind. For these infections the chronic current is characteristic.

At the heart of synchronisation the immunodeficiency, and also change of alternative or specific structure of activators during illness lays. The same factors cause propensity of opportunistic infections to generalisation, development septicopuemia.

Features of opportunistic infections concerns as complexity of treatment which is connected with plural stability of activators to the antimicrobial preparations, insufficient activity of factors of nonspecific protection, and also the weak immune answer of an organism of the patient to activator antigenes.

In this connection a major principle of treatment of opportunistic infections is application of preparations microbial actions and immunostimulation therapies.

At the same time opportunistic infections differ from the diseases caused by obligat pathogenic microorganisms, such epidemiological features, as a wide circulation in hospital hospitals, frequent cases endogenic infections.

In diagnostics of opportunistic infections microbiological methods of research are solving. The establishment of the activator (activators) of illness, definition enters
into their problem immunological the status of the patient, finding-out of a source and factors of transfer of activators.

In an establishment of an aetiology of disease the major importance has allocation of pure culture of the activator from a pathological material.

However allocation of culture of an is conditional-pathogenic microorganism from the patient yet does not confirm its participation in development of pathological process as the majority of is conditional-pathogenic microorganisms live at all or the majority of healthy people.

Therefore at diagnostics of opportunistic infections as the obligatory the quantitative criterion as which understand quantity colony cages of an allocated species of microorganism in 1 ml of an investigated material is provided.

**Bacteriemia** - the phase патогенеза infectious diseases during which time the activator gets to blood and is transferred with it to other places of localisation.

Thus its considerable part perishes, causing an intoxication, the others are grasped by cages limfoid makrofagal systems, perish or persistention in them. Activator reproduction in blood at bacteriemia бактериемии does not occur, as blood keeps the bactericidal properties.

The phase of bacteriemia is natural at the diseases transferred by blood-sicking parasites, and also at a belly typhus, leptospirosis, brusellosis, meningococcal infections, etc. It quite often complicates a current of heavy forms of the infections caused by is conditional pathogenic bacteria. In these cases bacteriemia passes in a sepsis.

Short-term bacteriemia it is possible at starvation, overfatigue, overheating, overcooling, the traumas, some medical interventions.

**Sepsis** - heavy sharp or chronic generalisation an independent infectious disease of blood. Usually develops against a deep immunodeficiency or an organism
sensitisation to activator antigenes. A unique place of dwelling and activator reproduction is blood.

Two forms of a sepsis - септицемию and септикопиемию allocate. At септицемии (a primary sepsis) the activator from entrance gate of an infection gets at once to blood and breeds in it.

Thus the primary local centre of an inflammation is absent at development of the secondary metastatic centres.

Septicopuemia (the secondary metastatic sepsis) develops as a result of generalisation of local infectious process. Depending on the primary local centre allocate patrimonial, wound, umbilical, urinegenital a sepsis.

A sepsis the peritonitis, a meningitis, polytraumas with a shock and the big loss of blood, infectious processes at newborns, older persons, blood disease, diabetes forms, AIDS, the period of many illnesses can become complicated. In the doctrine about a sepsis terms surgical, sepsis are often used.

For a sepsis, unlike bacteriemia, are characteristic loss by blood of bactericidal properties and, as consequence of it is activator reproduction in blood, a combination of signs of an infection, a microbic intoxication and the raised reactance of an organism.

An outcome of a sepsis the heavy. Lethality at the surgical form of a sepsis in 80th years reached 30-40 %, and at polymicrobial a sepsis and a sepsis at newborns was twice above.

According to the American authors, in the USA in 80th years from a sepsis annually died more people, than of taken infectious diseases all together.

The sepsis concerns to diseases. In an aetiology of the majority of forms of a sepsis the leading place occupies S. epidermidis and S. aureus, further follow E coli, Proteus spp., K. pneumoniae and other is conditional-pathogenic kinds of enterobacteria, and also P. aeruginosa, Streptococcus pyogenes, pneumoniae, faecalis; Bacteroides spp., Acinetobacter spp., Candida albicans, etc. Usually the sepsis is
caused by any one kind of a microbe. However approximately in 7-10 % of cases at a sepsis two are allocated or even three kinds of the activator.

Leading value in sepsis development belongs to an immunodeficiency, especially to inability of immune system to localise the activator in a place of the primary centre of an infection. The probability of development of a sepsis sharply raises at hit in blood of considerable quantities of the activator with high virulencity.

Shtams, allocated from blood sick of a sepsis, often carry to sick-lists ecological variations, possessing both high virulencity, and plural resistance to antibiotics.

Microbiological diagnostics of a sepsis consists in allocation of hemoculture and an establishment of the amazed link of immune system of an organism.

Isolation of culture from the primary and secondary local centres of an infection has auxiliary value.

The probability of allocation of hemoculture raises at a fence of a material prior to the beginning of chemotherapy, during temperature lifting, crops of considerable quantities of blood, a wide spectrum of selective environments and repeated crops of blood.

The choice of nutrient mediums and conditions of cultivation depends on a kind of the prospective activator.

Allocation from blood as obligat and is conditional-pathogenic bacteria irrespective of their quantity is regarded as a sepsis or bacteriemia.

**Pyoinflammatory processes**

Purulent or pyoinflammatory infections name the diseases accompanied by development of a purulent or serozno-purulent inflammation of a microbic aetiology.
Purulent infections can be sharp or chronic, local (local), system and генерализованными. Local and system divide in turn into some groups differing by origin, localisations and aetiologies.

**Aetiology wound and a burn infection**

The etiology structure wound infections depends on type and localisation of a wound, time and a place infecting.

At household, industrial, fighting wounds microorganisms get into a wound from a surface tools, the clothes, the damaged site of a skin and the bodies containing own microflora.

These is conditional-pathogenic microorganisms possess low virulencity both sensitivity to antibiotics and antiseptics.

During stay in a hospital hospital can occur infecting wounds other kinds of activators or the same kind, but other variant.

As a rule, microorganisms which have again got to a wound concern sick-lists ecological variations, they are steady against factors of nonspecific protection of an organism of the owner and antimicrobial preparations.

As a result there is a replacement hospital variants from a wound.

Activators of pyoinflammatory processes at wounds of a skin and soft fabrics at the first stage are golden staphylococci, is more rare - pyogenic a streptococcus, proteus, pseudomonase than a bacterium, enterobacteria, bacteroides.

In these cases in a wound monopopulations are quite often found out. At the subsequent stages the percent of the mixed infections increases, and frequent components of microbic associations become an intestinal stick, enterobacteria, proteus, pseudomonase bacteria, anaerobes.

Thus there is a change sensitive to antibiotics and antiseptics of bacteria on the polyresistant. At wounds, a small basin and a belly cavity with damages of internal bodies the quantity enterobacteria, pseudomonas and their associations increases.
Operational wound infections share on endogenic and exogenic, primary and secondary. At endogenic infecting activators get to a wound from a skin in the field of the operational field, the opened infectious centres and the bodies containing own microflora.

The specific structure of activators in this case corresponds to that of the operated fabrics and bodies.

The primary operational infection of a wound can result exogenic from activator drift at operative intervention. In these cases activators раневой infections become sick-lists shtams, circulating in the given branch.

Secondary wound the infection, as well as primary at a late stage of development, basically is caused by hospital variants of bacteria.

It has the mixed character with prevalence gram negative bacteria is more often.

The burn infection is in many respects close to wound. Infecting of wounds right after a burn occurs from the intact sites of a skin or a mucous membrane, from clothes, from air and other objects of an environment.

In a hospital the microflora is replaced with sick-lists ecological variations. Activators of a burn infection are staphylococci, a streptococcus, pseudomonas bacteria, an intestinal stick enterobacteria. At deep burns - anaerobic bacteria.

For a burn infection frequent presence at a wound of the several species of microorganisms, the expressed heterogeneity of their populations, high stability to antimicrobial preparations, constant change of specific and alternative structure of activators are characteristic.

The burn infection quite often becomes complicated a sepsis with high lethality.

Aetiology of pyoinflammatory diseases of various bodies and fabrics.

Activators of purulent infections are for the patient the kinds concerning to nonhospital ecological variants.
At exogenic infecting in hospitals purulent processes spicause nonhospital ecological variants pyogenic bacteria, in hospital hospitals - sick-lists ecological variants the same kinds. In an initial stage of illness the infection is caused by one kind more often, during the late period of illness the specific structure of activators extends basically at the expense of occurrence gramnegative sick-lists ecological variants.

Chronic processes in comparison with sharp and opened in comparison with the closed have wider spectrum of activators with presence of heterogeneous populations of sick-lists ecological variants.

To exogenic infections the widespread group of surgical diseases concerns: furuncles, abscesses, phlegmons, an erysipelatous inflammation, osteomyelitis and others.

Activators get into an organism through cracks, scratches, grazes, pricks, but can be brought ways from the infectious centres in other parts of a body, and also at the medical.

Interventions (an injection of medical products, biopsy, a blood sampling for the analysis etc.

The activator of the erysipelatous inflammation which are not accompanied by formation and allocation of pus, is a streptococcus.

The others nosological forms of infections polyetiologicly.

Leading activators are golden staphylococci, there is pyogenic streptococcus, an intestinal stick, proteus, bacteria, bacteroides, sometimes mycobacteria less often.

Initially closed processes usually are caused by population of one kind homogeneous for the signs, more often representatives of microflora of a skin of the organism.

After spontaneous or surgical opening often there is a superinfection sick-lists ecological variants the same kind or a secondary infection other kinds, more often non-negative bacteria (an intestinal stick, not purulent bacteria).
Abscesses and phlegmons are caused by sick-lists ecological variants the same kinds, as well as postoperative infections.

*Sharp purulent otitis* at adults various kinds staphylococci, pyogenic cause a streptococcus, in children - pneumonia streptococci, an intestinal stick, and also streptococci.

*Chronic average otitis* associations gramnegative bacteria (proteus, pseudomonas cause bacteria, anaerobes - fusobacteria, bacteroides).

*Sharp antritis* and фронтит cause staphylococci, streptococci, at chronic forms usually meet staphylococci and streptococci, proteus, klebsiella of a pneumonia, intestinal and pseudomonas sticks.

*Purulent parotitis* cause staphylococci, streptococci.

*Purulent* is complication of operative interventions on heart and lungs is more often. It is caused anaerobes by streptococci and sick-lists ecological variants of staphylococci.

*Purulent perykarditis* cause golden staphylococci, pyogenic streptococci.

*Postnatal mastitis and панариции concerns staphylococcal infections.* After opening the basic activator can join грамотрицательные bacteria.

*The purulent appendicitis* is caused by associations for intestines of bacteria: an intestinal stick, bacteroides, proteus and others enterobacteria.

*Cholecystitis* cause an intestinal stick, staphylococci and proteus.

*Purulent нанароккмум* is the mixed infection aerobic and anaerobic bacteria among which the leading part play an intestinal stick and bacteroides.

*The purulent peritonitis* results from infringements of permeability of bodies of a belly cavity at drift of microorganisms by from other bodies of the patient, and also during operative interventions and at wounds.

*Peritonitis activators at endogenic infections are associations of an intestinal stick, bacteroides, proteus, enterobacter, klebsiella, a fecal streptococcus, is frequent*
with staphylococci. The postoperative peritonitis is caused by sick-lists ecological variants staphylococci, an intestinal stick and others gramnegative bacteria.

*Sharp the osteomyelitis* is caused golden staphylococcus, by chronic and traumatic forms of illness - associations staphylococci with gramnegative bacteria.

*Omfalit* develops usually in the first 10 days after a birth of the child as a result infecting umbilical wound epiderm staphylococcus, also синегнойной and intestinal sticks.

*Adnecsit* divide on specific - gonococcal, tubercular, and nonspecific - opportunistic, caused by an intestinal stick, staphylococcus, streptococcus.

**Microbiological diagnostics.**

For an establishment of the activator (activators) of a purulent infection the major importance has bacteriological research.

For the preliminary diagnosis use immunofluorescence a method. At not clear or negative results of bacteriological research define increase титра antibodies to dominating culture or diagnosticum from which kinds mainly meet at defined nosological illness forms.

The conclusion about the activator is given on the basis of quantitative criteria. In case of allocation of a monoculture from the closed purulent centres etiology significant are $10^4$CU (colony units), at open processes - $10^5$CU.

When it is allocated two or more kinds, the diagnosis mixinfection is made. It is recommended to repeat bacteriological researches every 5-7 days as the structure of activators during an infection can change.

**Aetiology of opportunistic bronhopulmonary infections.**

Infections of respiratory ways can be caused specific activators and is conditional-pathogenic bacteria.
The last proceed in the form of a bronchitis, a pneumonia, an abscess and a lung gangrene, a pleural cavity.

The leading place in the given group of illnesses is occupied with a sharp and chronic bronchitis.

Infected with activators of opportunistic infections occurs air-drop by.

In some cases activators get from blood. It takes place at operative interventions, endoscopical researches, during time intratrakheal introductions of aerosols and solutions.

Drift of is conditional-pathogenic bacteria in respiratory ways not necessarily leads to infection development. At healthy people bronchial tubes possess the expressed ability to self-cleaning.

Besides, the mucous membrane of a respiratory path possesses the expressed barrier functions against is conditional-pathogenic bacteria.

Therefore the high infecting dose of the activator, infringement of integrity is necessary for infection occurrence mucous and decrease in self-clearing function of respiratory ways.

Immunodefinitions conditions of a different origin substantially raise risk of occurrence of an infection.

The aetiological structure bronkheal diseases appreciably depends on their form. A sharp bronchitis in many cases originally results from virus infections.

In some cases aetiological agents streptococci of a pneumonia, a bacterium influenza can be primary, etc. a virus infection can join secondary bacterial, the fungoid infection therefore process gets purulent character is more rare.

As activators of secondary infectious agents meet is more often: aureus, H. influenzae, S. epidermidis, E coli, Klebsiella of pneumoniae, etc.

The chronic bronchitis in an initial stage proceeds as noninfecting which in the subsequent passes in the infectious process caused by various microbic associations.
Leaders from them are streptococci of a pneumonia, a bacterium influenza, golden staphylococci. Depending on variety of factors activators of a chronic bronchitis can be an intestinal stick, klebsiella of a pneumonia, proteus, pseudomonas bacteria, a streptococcus, neisseria, enterobacter, bacteroides, fusobacteria, Candida, etc.

Qualitative and quantitative structure of microbial associations constantly varies in the course of an infection.

At a finding of the patient in a hospital hospital leading value in an etiology is got by sick-lists ecological variations is conditional-pathogenic bacteria.

At an abscess of the lung arising as complication of a pneumonia or a purulent bronchitis, leading value occupy pus cocci in association with gram-negative bacteria, and at a lung gangrene – anaerobical nonsporing bacteria in association with pus cocci and gram negative bacteria.

The sharp pneumonia is caused pneumoniae in a monoculture or in association with golden or staphylococci and gram negative bacteria.

About 10-30% of a pneumonia at children - viruses and mycoplasmas. Along with the mentioned bacteria activators of hard proceeding pneumonia at children can be Pneumocysta carinii, and at adults - Legionella pneumophilia (illness of legionaries).

The chronic pneumonia in comparison with a sharp thicket has a polymicrobial etiology, and number of associations in the qualitative and quantitative relation much more. Its activators are the same kinds of bacteria which meet at a chronic bronchitis and a sharp pneumonia.

As a chronic pneumonia proceeds pulmonary mycobacteriosis, caused is conditional-pathogenic mycobacteria.
**Microbiological diagnostics.**

The definitive diagnosis establish by carrying out of bacteriological research for the purpose of allocation of pure culture (cultures) of the activator.

Preliminary or additional data can be received by means of reaction immunofluorescence and reactions of swelling of a capsule, increase revealing титра antibodies during illness to dominating autoculture.

Etiologocly for sputum and washing waters of bronchial tubes following minimum concentration of activators are significant: for streptococci - $10^6$CO/ML, staphylococci - $10^5$, enterobacteria and gramnegative nonfermenting bacteria - $10^4$, Candida albicans - $10^3$.

At etiologocly significant cultures (populations) define sensitivity to drugs, to antiseptics and, in case of need, virulencity and alternative structure (it is grey - fago - rezistens-biovary).

As in the course of illness change of specific and alternative structure of activators each 5-7 days repeat research is often observed.

**Aetiology opportunistic urinoinfections.**

Opportunistic urinoinfections proceed in a kind nephritis, a pyelonephritis, abscesses, a cystitis, a prostatitis, urethritis, a postoperative infection, including connected with change of kidneys.

The current of the listed local infections quite often becomes complicated an urethral fever, urinosepsis and sometimes a bacterial shock.

Long allocation with urine of considerable quantities of bacteria in the absence of clinical displays is designated as asymptomatic bacteriauria.

Is conditional-pathogenic bacteria get into uric system by, at traumas of bodies of urinogenital system, their contact to the infected bodies of a small basin and ascending by through an urethra.
Last way is the main thing. It can be result of medical interventions or occurs spontaneously. The destiny enter in urinogenital system of is conditional-pathogenic microbes depends on an infecting dose of the activator and especially from a condition of local and general immunity.

Risk categories are patients with congenital developmental anomalies of uric system, infringements of conductivity of a spinal cord, the infectious centres in a small basin, an immunodeficiency, medical interventions on urinogenital system.

Nephritis it is usually caused by S. pyogenes, and also staphylococci.

The others urinoinfections are caused mainly gramnegative bacteria, first of all E.coli and Proteus spp. Into the list of activators also enter K.pneumoniae, Enterobacter spp., Citrobacter freundii, P.aeruginosa, S. epidermidis, S. aureus, Streptococcus pyogenes et faecalis, Bacteroides spp., Candida.

Urinopathogenic intestinal sticks on which the majority urinoinfections is necessary, concern to defined serological variations, contain R-adgeziny to receptors uric ways.

They form a capsule, allocate гемолизины and well breed in the sour environment. Sharp urinoinfections are caused by one kind, chronic and postoperative - association of activators more often.

**Microbiological diagnostics** opportunistic urinoinfections just as asymptomatic bacteriuria, it is based on culture allocation. Rough data in the activator can be received microscopy of a deposit of urine, additional data - with the help serological reactions with autoculture significant kinds.

The estimation of results of researches is difficult. Urine at passage through urethra department, as a rule, representatives of normal microflora, the majority from which simultaneously are also the main activators opportunistic urinoinfections.

For difference of the activator urinoinfections from microbes-kontaminantov and others etiology insignificant kinds use a quantitative method.
The maintenance of the allocated culture in number of $10^6$ and more individuals in 1 ml of urine certainly specifies on этиологическую a role of the allocated culture. The size $10^5$ in most cases also is estimated as etiology the significant.

Sizes $10^4 - 10^3$ can contain in urine healthy and the more so sick people, and consequently reception of additional criteria (repeated seeding, a culture accessory to sick-lists ecological variations, pathogenicity, etc.) and a joint estimation with the urologist demand.

The diagnosis asymptomatic бактериурии is exposed when in the absence of symptoms of defeat of urinogenital ways from urine repeatedly allocate the big number of bacteria ($10^6$ and more).

**Aetiology of opportunistic sharp intestinal infections.**

Opportunistic acute intestinal disease (AID) cause Escherichia coli, Citrobacter freundii, Klebsiella pneumoniae, Enterobacter cloacae, aerogenes, etc., Serratia marcescens, Hafnia alvei, Arisona, Proteus mirabilis, vulgaris, etc., Morganella morganii, Providencia rettgeri, stuarty, alcalifaciens, Pseudomonas aeruginosa, Campilobacter jejuni, Plesiomonas shigelloides, Vibrio haemolyticus, Staphyloccocus aureus, Streptococcus faecalis (enterococcus), Clostridium perfringens, Bacillus cereus.

The diseases caused by specified kinds, proceed as food toxicinfection is more often, is more rare - a microbiic intoxication (staphylococcalriosis, clostridial) and an infectious disease (escherichiosis, campilobacteriosis).

Acute intestinal disease occurs as a result of reception by kontaminirovan th microbes of food to which they get from people - patients and carriers, is more rare from animals. In foodstuff the specified kinds of bacteria are capable to reproduction in the conditions of a room temperature, and a pseudomonas and klebsiella - at temperature of a household refrigerator.

At reproduction staphylococcus and clostridia in foodstuff collects exotoxin.
Except the alimentary transfer of activators kontakt household by and through water is possible, but these ways are less effective, as in most cases cannot provide hit in an organism of a sufficient infecting dose.

Except hit of a high infecting dose and virulencity the activator in development of disease the conditions promoting fast and its mass reproduction in a thin gut or a stomach have great value.

At staphylococcus and clostridia a pathogenicity primary factor is exotoxin, at other microbes - endotoxin which is allocated in considerable quantities at mass disintegration of the bacteria which have got to intestines and local and general damaging an effect has.

Thus clinically diseases are shown in the form of a gastritis, an enteritis, gastroenteritis, колита more often.

The majority of activators is caused by the diseases proceeding in the easy form. E coli can cause the heavy forms accompanied by dehydration of an organism or becoming complicated sepsis.

Clostridia perfringens cause the diseases proceeding as intoxications, a sepsis, an enteritis.

Microbiological diagnostics opportunistic acute intestinal disease is based on bacteriological research. At chronic and long forms in blood whey quite often reveal increase титра antibodies to dominating culture.

On conditional pathogenic bacteria spend research after negative results of crops on obligat pathogenic activators. It is more expedient to sow a material on the differentsiial diagnostic environments allowing along with obligat pathogenic bacteria to allocate is conditional pathogenic kinds.

The estimation aetiological roles of the allocated cultures is spent on the basis of their conformity to the criteria listed above.

For the bacteria, present at intestines of healthy people, the size of $10^6$ CU/G materials is significant.
For allocation of an intestinal stick and bacteroides which in intestines are present at significant amounts, the further researches are necessary.

At the diseases proceeding as food poisoning, reliability of the diagnosis raises at detection of the same culture in foodstuff.

**Activators opportunistic mycobacteriosis.**

*Mycobacteriosis* - the diseases connected with is conditional-pathogenic mycobacteria. On a clinical picture these diseases are similar to a pulmonary tuberculosis.

However meet and nonlung forms. Unlike the tuberculosis activator is conditional-pathogenic (not tubercular) mycobacteria cause an opportunistic infection.

Among activators mycobacteriosis most often meet **M. avium**, **M. avium** complex, **M. kansasii**, **M. fortuitum**, **M. chelonae**, **M. marinum**, **M. ulcerans**. To the morphological and physiological signs they are similar to others mycobacteria, that is are fast acid polymorphic sticks which are cultivated on Levenshtejn-Iensen environment.

On pigmentforming, to character and growth rate them divide into four groups:

1- Photochromogenic mycobacteria which at cultivation on light get a yellow-orange pigment. Them concerns **M kansasii**;

2- Chromogenic mycobacteria which slowly grow on nutrient mediums, forming a bright orange pigment irrespective of light presence. Them concern **M scrofulaceum**, **M gordonae**;

3- Nephchromogenic mycobacteria which cultures nonpigment or have a weak orange shade. Representatives of this group are микобактерии the MAS-COMPLEX;
4- Fast-growing mycobacteria which cultures can be received within one week. It M.smegmatis, M.fortuitum, M.phlei.

**Laboratory diagnostics** of mycobacteriosis is spent by a bacteriological method by crops of an investigated material on Levenshtejn-Jensen environment.

Identification of the allocated culture is spent on numerous cultural, to biochemical and other tests, including on growth in different temperature ranges from 22 ° to 52°C and definition amidase activity.

For diagnosis acknowledgement «mycobacteriosis» repeated allocation same shtamms the activator is necessary provided that mycobacteria of a tubercular complex have not been found out.

Opportunistic mycobacteriosis it is necessary to distinguish from secondary infections which develop at sick of chronic diseases, oncological patients, at wrong reception of antibiotics and other adverse conditions.

Especially often secondary mycobacteriosis develop at sick of AIDS not an immunodeficiency background that has given the chance to name their AIDS-assotation or AIDS-display infections.

In comparison with tubercular микобактериями activators opportunistic mycobacteriosis as a whole are less sensitive to antitubercular chemotherapeutic preparations.

For treatment of these diseases are recommended: rifampicin, streptomycin. The individual choice of antibiotics is Thus necessary.

**Activators of opportunistic mycoses.**

The group of mycoses which are caused by is conditional-pathogenic fungi from sorts Aspergillus concerns them, Candida, Mucor, etc. Some of them, for example Candida, are a part of normal microflora of a human body.
However, more often they complicate such diseases as diabetes, an immunodeficiency, including AIDS, and also show the pathogenic action in associations with is conditional-pathogenic bacteria at the persons receiving antibiotics of a wide spectrum of action, especially tetracycline, cytostatics and beam therapy.

The infection arising thus can be local (Aspergillus lungs, Aspergillus an otitis, an oral cavity candidiasis) and generalisation (Candida a sepsis). In any case the macroorganism condition has defining an effect on character of development of infectious process.

The in itself mentioned fungis, as a rule, cannot cause a monoinfection. Usually they infectious process in associations with is conditional-pathogenic bacteria.

Along with is conditional-pathogenic bacteria in such microbiosinosisis can be present saprophytus and is unconditional-pathogenic kinds.

For realisation of pathogenic potential microbiosinosisis, including containing is conditional-pathogenic fungi, the corresponding conditions consisting available of an immunodeficiency are necessary. In norm carrier is conditional-pathogenic fungi in cavities and on mucous membranes in defeats interfere with transition mechanisms antyfungal organism protection.

Allocate three levels of the morphological structures which are carrying out a protective role. Epithelial level participates in retseptor-ligandnyh interactions, between receptors epitheliacytes of fungi, providing adhesion with the subsequent colonisation of mucous membranes.

Thus it is necessary to mean, that receptors of epitheliacytes in norm are occupied by representatives of normal microflora.

Therefore fungi, enter from the outside in defined biotops an organism, should possess antagonistic properties in relation to the given microflora to release
corresponding loci for adhesion and the subsequent invasion the activator in depth of fabrics.

Thus, display by is conditional-pathogenic fungi of pathogenic properties in structure of microbiosinosis is connected with their ability to adhesion, colonisation, and is indispensable with immunodeficiency presence in a human body amazed with them.

An exception make yeasts like fungus of Candida which as representatives of normal microflora initially are present at a human body owing to what for manifestadde endogenic infections they require only occurrence immunodefition conditions in an organism.

Early diagnostics of opportunistic mycoses is rather difficult. It is spent by complex microbiological, immunological and patomorphological the researches, allowing to make a certain notion about an outset of infectious process.

**Candidiases.** Activators of candidiases are various kinds of Candida. Most often disease causes C.albicans.

**Morphology, physiology.** Yeasts like fungus of Candida - fungus lost a phase and fixed in haploid a condition. They are close to yeast. In a pathological material and in cultures form oval budding barmy cages and pseudomycelium. C.albicans well grows on usual nutrient mediums at 20°C and 37°C.

On the basiscultural, microscopic and biochemical characteristics differentiate more than 100 kinds of sort Candida, from which only the few (less than 10) cause candidiases (C.tropicalis, etc.).

**Antigenes.** Glicoproteins cellular walls yeasts like fungi of Candida define their specific antigen specificity. C.albicans subdivide on 3 serological variations, and yeasts like fungus of Candida has the general antigenes.
**Pathogenicity.** Pathogenicity factors Yeasts like fungi of Candida are hemolisin, lipids, polysacharites, some hydromanholes, endotoxins.

**Pathogenesis and immunity.**

Yeasts like fungus of sort Candida cause the various sharp and chronic infections having local character. Diseases can develop in the form of primary or secondary infections as a result exogenous or endogenous infecting.

Widespread the candidiasis of an oral cavity typical for newborns, and also for the persons, suffering heavy diseases of a different aetiology (malignant tumours, etc.).

Besides, quite often meet candidiasis vulvavaginit, developing in the pregnancy period, at a diabetes; a candidiasis as a secondary infection against a cancer of lungs, illnesses, AIDS and other diseases; a candidiasis, at which defeats are localised in large folds of a skin (under mammary glands) or on hands at their frequent humidifying; a chronic candidiasis of a skin and mucous membranes.

In connection with development of the microbiological industry and, first of all, manufactures of the fiber based on use yeasts like fungi of fungis of sort Candida, the number of allergic diseases has considerably increased in corresponding regions of our country.

Allergen in this case is glicoproutin, conditionally named paprin, getting into an organism through respiratory ways.

More rare defeats of the person concerns canidial endocarditis.

More often candidiases arise and develop at persons, is long accepting antibiotics of a wide spectrum of action and hormonal preparations, with the expressed infringements microbic биоценозов oral cavities, intestines, and also immune system of an organism.

At candidiases antibodies of classes IgG, IgM, IgA collect.
Ecology and epidemiology.

Various kinds Candida widespread in the nature, and C.albicans is the representative of normal microflora of a human body. They are steady enough against influence of factors of environment.

Laboratory diagnostics.

Spend microscopic, cultural, biochemical and serological researches. Microscopy a pathological material with a view of detection of budding barmy cages or elements pseudomycelium. Cultures from a pathological material receive on Sabouraud dextrose agar or a mash-agar at 20 and 37°C.

Fungi of Candida for 2-3rd day after crops form small convex colonies which merge in the powerful formations growing into a nutrient medium.

Identification of fungi of sort Candida spend on the basis of data of microscopy of a pathological material, cultural signs, biochemical activity, growth types filamantation.

Formation of threads (filamantation) occurs for the account pseudomycelium which differs from true that has no general cover and partitions, and consists of long and thin cages. Pseudomycelium arises by consecutive lateral or trailer budding.

Serological diagnostics spend by means of agglutination reactions, complement fixation test, presipitation assay, immunoenzime assay, etc. Allergic tests apply various allergens.

Preventive maintenance and treatment.

As specific prophylactics always use the killed vaccines (including prepared).

Chemotherapeutic means apply antibiotics (nystatin, etc.), some derivatives имидазола, 5-flutsitozin.
**Aspergillosis.**

It is known 150 kinds and subspecies of aspergillus. Many of them are pathogenic for the plants, some insects and pets. For the person most n kind Aspergillus fumigatus though also other kinds of this sort can cause diseases.

**Morphology and physiology.**

Aspergillus concern to called filamentous fungi. At growth on Sabouraud dextrose agar environment or a mash-agar at 20°C they form septicum mycelium with conidia, bearing sterigma and conidiospores grey, green, blue-green, black or other colours. In fabrics, excudate, sputum aspergillus come to light in a kind septicum a floccus.

Aspergillus - strict aerobe, well grow on various nutrient media at pH 6,0-6,5. Optimum sources of carbon are олиgosахариды, nitrogen sources - the salts, some amino acids, etc. A. flavus is toxigen , capable to form exotoxins (flavotoxins) in foodstuff.

Antigenic properties aspergillus are a little expressed, however disputes and мицелий fungis can be allergens.

**Pathogenicity.**

Conidia cages of aspergillus can cause corresponding aspergillosis defeats of the injured cornea, the burnt fabrics, wound surfaces.

Pathogenicity factors are some hydromanholes, not albuminous exotoxins, etc.

**Pathogenesis.**

Aspergillosis develop mainly at persons with immunodefition conditions or with it is appreciable the broken stability to an infection against a leukaemia,
tumours. Normal macrophages can grasp conidia and promote dissemination of fungi in an organism, and floccuses are sensitive to pernicious action natrophylis and monocytis.

Aspergillosis can be invasion (pulmonary), noninvasive and allergic (asthmatic).

Immunity.

At the people infected by aspergillosis, can develop HST; in blood whey antibodies of classes IgG and IgE come to light.

Ecology and epidemiology.

Aspergillus widespread in the nature worldwide. They are steady in an environment.

Cases of aspergillosis are described in various regions of Globe. Specific preventive maintenance is absent.

Aspergillosis, especially pulmonary forms against a developing allergy difficultly give in to treatment, that in one cases leads to a pneumonia, in others - to a bronchial asthma.

Laboratory diagnostics.

Spend microscopy of sputum on presence septicum of a floccus or "stoppers" from mycelium. Can come to light conidiaphore with conidia.

Receive also pure cultures at crops of a pathological material on Sabouraud dextrose agar or a mash-agar. Put some serological reactions, for example immunodiffusion, or for revealing IgE spend radioallergosorbent the test.
**Zygomycosis (mucormycosis).**

Zygomycosis are caused by the lowest fungi from a class zygomycetes. Thus it is the most frequent among them meet Rhizopus and Micos.

Fungi are cultivated on usual nutrient mediums on which the colonies darkening in fructification are formed.

As entrance collars of an infection respiratory ways serve and the injured sites of a skin and mucous membranes are more rare.

Ficomysetes amaze the people, suffering with a diabetes, лейкозом, lim-fomoj and other heavy chronic diseases, with the weakened immune system of an organism more often. At germination in walls of blood vessels фикомицеты cause thromboses (for example, in nosal sine, easy, a gastroenteric path).

Separate kinds of Ficomysetes possess the active fermental complexes accelerating reactions of curling of blood.

**Laboratory diagnostics** spend by microscopic and cultural researches. In a pathological material comes to light nonsepticum mycelium.

**Specific preventive maintenance** is absent.

From chemothepathic means recommend amphoterisinum B, 5-flutsitozin, some derivatives imisadol, etc.

**Pneumocystosis.**

The activator pneumocystosis, or pneumocystosis pneumonia, is Pneumocystis carinii which concerns group blastomyces, i.e. budding barmy microorganisms.

**Morphology and physiology.**

In sputum and a pulmonary fabric (biopsy) it is possible to reveal three stages of development P. carinii: cystis (pneumocystis).
Cystis settle down in a pulmonary fabric or in respiratory secrets. In them contains to 8 oval intracystis structures (little bodies). Sometimes out of cystis are found out trophosoits which settle down groups in association with cystis.

Cystis oval under the form, from 3 to 5 microns in diameter, are an intermediate stage between trophosoits and cystis.

Irrespective of a development stage in painted histologic preparations P. carinii happens it is presented by single or budding cages.

In pure culture on nutrient mediums the given microorganism is not received yet owing to what there is no information on its possible stages of development in vitro, and also about antigen properties.

Pathogenicity.

P. carinii - an is conditional-pathogenic microorganism. At people with normal immune system the infection is not shown.

At immunodeficiencies the pneumonia (pneumocystosis) - the main reason of death rate of the persons, suffering AIDS develops pneumocystis.

Pathogenesis.

At newborn and more senior children disease proceeds in two forms: epidemic and sporadic. The first develops slowly within 4-6 weeks and in 20-50 % of cases comes to an end lethality.

Sporadic pneumocystosis children suffering agamma - or gammglobulinemia is more often are ill. Contrary to the epidemic form in these cases the sharp beginning is marked.

Disease lasts months before there will come the respiratory insufficiency which is coming to an end in 100 % of cases by a lethal outcome.

Pneumocystosis can develop at the persons receiving иммунодепрессанты at treatment of a cancer, illness and others or at transplantation of bodies.
Pneumocystis pneumonia develops more than at 80 % of the persons, suffering AIDS. At 60 % of such patients pneumocystosis is an initial opportunistic infection with a lethal outcome.

**Ecology and epidemiology.**

The fungi widespread in the nature, comes to light at rats, mice, dogs (usually without disease display). The latent infection caused P. carinii, it is extended among people. It is supposed, that the infection develops as a result of inhalation cystic forms of a parasite. Through foodstuff and water the infection is not transferred. P. carinii it can be transferred transplacentary.

**Laboratory diagnostics.**

The diagnosis pneumocystosis can be put only at indisputable identification P. carinii in a pathological material.

Pneumocystosis proceeds in the form of a monoinfection or the mixed infection, for example with cryptococcosis, cytimegalovirusis an infection, a tuberculosis.

For colouring P. carinii in a pathological material use a paint Romanovsky-Giemza or akridin the orange.

**Serological diagnostics.**

P. carinii it is possible at use of an indirect method immunofluorescence and a so-called ELISA-method (*enzime-linked immunosorbent assay*). Thus special difficulty consists in differentiation of the antibodies which are available for healthy people of control group, from the antibodies appearing a pneumonia.

**Preventive maintenance and treatment.**

Specific preventive maintenance pneumocystosis pneumonia is absent. From chemotherapeutic means are recommended pentamidin isopionat, kotrimaxazol, dapson in a combination in tremetoprim, etc.
Same medical products are recommended and for chemoprophylaxis of pneumocystosis at patients AIDS.

**HOSPITAL (YATROGENICAL) INFECTIONS**

In hospitals of the world millions patients annually arrive. The main task of collectives of these establishments is fast recovery of health of the hospitalised patients and creation of safe conditions of their stay in these hospitals in respect of possible infection with an hospital infection.

Cases of development of diseases or boundary conditions with them as a result of medical aid rendering were known to doctors for a long time.

Without reason one of the most ancient medical precepts says: Primum the priest nocere (first of all do not do much harm).

In the subsequent the illnesses connected with medical intervention, medical aid which appears the patient in hospitals, out-patient-polyclinic establishments and house conditions have received the name yatrogenical (from Greek word yatros - the doctor, genius - an origin).

Yatrogenical (hospital) infections are infectious diseases of the hospitalised patients during their stay in hospitals or at medical workers in an operating time in them.

There are they worldwide and it are a serious problem for treatment-and-prophylactic establishments of health protection.

Hospital infections, as a rule, join the basic diseases or for the first time arise at newborn children.
Distinguish such basic groups nosological forms of an hospital infection:

- generalisation forms (bacteriamia, a sepsis, septisemia);

- wound postoperative infections and postinfectious complications, pyoinflammatory complications of wounds and burns;

- sharp intestinal, respiratory, urogenetal, transfussion infections;

- the diseases caused by long-term treatment by antibiotics, hormones.

Yatrogenical infections should be distinguished from at what infection of the person occurs in nonhospital conditions, and illness is shown after a while after receipt of the patient in a hospital concerning other disease.

Yatrogenical infections have arisen during those far times when the first surgical operations have been performed. In XVII, XVIII and first half XIX century a postnatal septic fever developed at 50-60 % of patients, giving almost 100 % lethality. An establishment of the microbic nature wound and postnatal complications (L.Pasteur) and working out of methods of antiseptics (N.Pirogov, D.Lister), and then aseptic and others epidemiological actions have led to sharp reduction of number yatrogenical infections.

In the end of XIX and first half XX century yatrogenical infections were registered only at 3-5 % of the hospitalised patients.

The new period of increase of number yatrogenical infections has come in the early fifties XX century and proceeds on present time. For it the sharp increase in frequency and weight of these infections, their distribution in medical institutions of all profiles, expansion of specific structure of activators and nosological forms of diseases is characteristic.
The reasons of the developed situation are various. It is necessary to carry the following to them:

1. Unfairly wide, often irrational application of antibiotics which has led to distribution множественно forms of bacteria steady against chemotherapeutic preparations. There is a formation and selection «hospital strains» the microorganisms possessing high virulencity and plural medicinal stability.

2. Increase among the population of groups of the raised risk connected with wide introduction in medical practice of methods of diagnostics with infringement of integrity of skin and mucous covers, expansion of a spectrum and weight of operative interventions, frequent use of the medical products suppressing immune system, increase in population of people of persons of elderly and senile age and increase of quantity infectious and noninfectious.

3. Expansion of circulation of microorganisms in medical institutions, is absent the control over their circulation. Increase in number of contacts to medical workers and objects of the hospital environment, contamination microorganisms. By the way, considerable frequency carrier pathogenic microflora / e.g., golden staphylococcal/reaches 40%.

4. Infringement of rules aseptic and antiseptics, a deviation from sanitary-and-hygienic norms for hospitals.

Yatrogenical infections worsen indicators of medical aid to the population, Narrow possibilities of hospitalisation of patients, cause in the population mistrust to activity of medical workers, lead to huge labour losses and the big additional financial expenses on social security.
Hospital-acquired infections: definition of the term

- Infections which develop in patients due to physical manipulations for example, invasive procedures (intravenous cannulation, urinary cauterisation, surgery, etc) which are carried out in:
  - hospital
  - ambulatory
  - at home

Hospital-acquired infections: the reasons for their spreading

- The widespread and frequent use of therapeutic and prophylactic antimicrobial agents provides selective pressure for the proliferation of drug-resistant microorganisms.
- Increase of the number of invasive methods of therapy and medical tests:
  - diagnosis that is accompanied by breakdown of physical barriers such as skin and mucous membranes,
  - surgery,
  - use of the immune suppressive drugs,
  - aging of the human population, survival of immune compromised persons,
  - high frequency of non-infectious underlying chronic diseases.
- Changes which occur in the hospital environment:
  - larger inocula of opportunistic pathogens,
  - increase in the number of visits the hospitals by patients.

Hospital-acquired infections: aetiology

- Opportunistic microorganisms
- Highly pathogenic microorganisms:
  - Hepatitis B virus
  - AIDS
Influenza
Viruses which cause acute respiratory and enteric infections
Salmonellae and Escherichiae in children
Adenoviruses (conjunctivitis)
Herpes and Cytomegalovirus infections
Chlamydia and Mycoplasma (urethritis)
Dermatomycosis

**Hospital-acquired infections: clinical ecological variants of pathogens**

The pathogens are characterised by the next properties:
- highly resistant to numerous antimicrobial agents,
- highly resistant to antiseptics and disinfectants,
- highly resistant to the factors of innate immunity of the human organism.

**Hospital-acquired infections: condition for their development**
- Infection which is developing due to the medical manipulations
- Debilitation of hospitalised patients: their susceptibility to the infection

**Hospital-acquired infections: specificity of pathogenesis**
- Clinical manife usually patients are debilitated or immunosuppressed.
- Even in normal the immune response developed against opportunistic microorganisms is lower then one formed against high pathogenic microbes.
- In the course of the disease immune deficiency is getting even higher (the results are generalisation of the infectious process or development of chronic disease). station and the composition of pathogens are due to the site where the invasive diagnostics or corrective, and maintenance procedures where applied.

**Hospital-acquired infections: diagnosis**
The diagnosis includes:
- investigation of the:
- patient
- medical personnel and other patients as they are possible sources of the infectious agents
- Hospital surroundings as they are possible factors of transmission of the pathogens

The diagnosis hospital-acquired infection could be stated when:
- hospital variant of etiologic agent has been isolated from the patient (even if the source of the infections and the factors of transmission where not found)
- When infectious process appeared after the contacts with hospital personnel after the period of time necessary for incubation period was finished (it is equal to 2-3 days in the case of opportunistic infections)

**Hospital-acquired infections : prophylaxis**
- Examination of patients and hospital personnel in connection with possible carriage of the agents of hospital infections.
- Examination of hospital living areas and therapeutic preparations to reveal their possible contamination with hospital opportunistic and iatrogenic pathogens.
- Maintaining a clean, disinfected environment.
- Treatment of physicians and other hospital personnel who are shown to be carriers of potential pathogens with specific chemotherapy to eliminate the carrier state.

*The aetiology yatrogenical infections* is characterised as the general for all infections, and specific signs.

Continuous change of structure of activators and their relative density concern them in development of infections. These changes lead to formation and a wide
circulation in hospital hospitals special ecological variants the activators named hospital.

They differ from nonhospital plural resistance to the antibiotics, the lowered sensitivity to antiseptics, high polymorphism of populations, concerning high stability to competitive action constant microflora, wide and вариабельным to sets of factors - virulencity, the expressed ability to colonisation of a skin and mucous membranes.

Sick-lists ecological variants of bacteria are formed from внебольничных under the influence of factors of the hospital environment.

Unlike nonhospital they are well adapted for dwelling and disinfecting actions spent in the hospital environment (from here the term ecological variants). Along with it they are steadier against nonspecific factors of protection, than nonhospital strains.

Along with sick-lists ecological variants of activators yatrogenical infections are obligat-pathogenic bacteria, anaerobic bacteria and the viruses causing a hepatitis In, a HIV-infection, a flu, sharp respiratory and intestinal virus infections, local and generalization forms herpes infections, and also chlamydia and mucoplasma infections and many other things.

However activators big parts yatrogenical infections are is conditional-pathogenic bacteria. These are sick-lists and nonhospital ecological variants.

It is the big and diverse group in the regular relation, causing in the person of illness under certain conditions.
Is conditional-pathogenic microorganisms meet among bacteria, mycoplasma, fungi, the elementary. Some viruses, for example, alpha-herpesvirus 1 and 2 types, beta-herpesvirus to many signs are close to them.

In a modern pathology of the person it is supposed etiological a role about 100 kinds of is conditional-pathogenic microorganisms.

**Among them representatives of following sorts have major importance:**

*Staphylococcus (S. aureus - to 60 % of all cases hospital infections), Streptococcus (Str. Pyogenes, Str. Pneumonia), Peptostreptococcus, Escherichia, Enterobacter, Klebsiella, Citrobacter, Serratia, Proteus, Pseudomonas, Haemophilus, Acinetobacter, Bacteroides, Bacillus, Mycobacterium, Mycoplasma, Candida, Cryptococcus, Pneumocysta.*

In the ecological relation is conditional-pathogenic microorganisms are non-uniform. Among them there is a group living the kinds which inhabitancy is foodstuff, soil, water, an organic waste of activity of people, medical products.

The majority of these kinds are capable to live in different biothops a human body and under certain conditions to cause diseases (saphronosis).

However for kind continuation the live environment for them is not obligatory.

In hospital hospitals from the given group of microbes meet legionella, sarsina, proteus, klebsiella of a pneumonia.

Some kinds of parasites of animals, for example, conditional-pathogenic mushrooms in cavities and on mucous membranes in defeats interfere mechanisms antifungal organism protection.
Infection of people with sick-lists ecological variants occurs basically экзогенно as a result of medical interventions and activator penetration contact or aerosol by in operational and dressing premises.

They are brought by the same ways in the burn and traumatic wounds, the open inflammantory centres, fabrics, cavities and paths with the broken integrity of a mucous membrane.

Infection of people with sick-lists strains also occurs through defects of a skin and mucous membranes by autoinfectious from places носительства (a nose, a nasopharynx, hands, hair).

Sick-lists ecological variants is conditional-pathogenic bacteria are representatives microflora of the organism, and in a case sapronosis are inhabitants of environment much less often.

Possibility of their reproduction on objects of an environment is complicated, and experience terms in it are limited. In case of scrupulous performance of antimicrobial actions objects of the hospital environment can be shown to safe quantities.

Yatrogenical an infections caused nonhospital ecological variants, basically concern to endogenous.

They arise at drift of a considerable quantity of representatives of normal microflora in the internal environment of an organism through the damaged skin and mucous membranes, especially against decrease in intensity of natural immunity and suppression of ability of formation of the effective immune answer to activator antigens.

In development yatrogenical the infections caused by is conditional-pathogenic microorganisms, the main role belongs to medical interventions.

The nosological form and structure of activators depend on type and localization.
The basic nosological forms of the hospital an infection.

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<tr>
<th>Generalized forms</th>
<th>Sepsis, meningitis, osteomyelitis, pneumonia in maternity homes, newborn departments, reanimation departments.</th>
</tr>
</thead>
<tbody>
<tr>
<td>The diseases of a skin and subcutaneous fat</td>
<td>Pyoderma, abscess, conjunctivitis, otitis, mastitis, endometritis, wound postoperative infectious, post injection complications.</td>
</tr>
<tr>
<td>Acute clinical infectious</td>
<td>Gastroenteritis, enteritis</td>
</tr>
<tr>
<td>Urinary diseases</td>
<td>Urethritis, cystitis, pyelonephritis</td>
</tr>
</tbody>
</table>

The characteristic of activators of the hospital infection:
- Septic infections,
- Diarrheal diseases,
- Usual infectious diseases.

Decoding of the etiology of the hospital infection:
* Microbiological research of a pathological material received from the patient with use of methods of the quantitative account of microorganisms in an examined material.
* Identification and differentiation of the allocated microorganisms.
* Definition of a spectrum of sensitivity of the data of strains to antibiotics.
* On occasion: definition in dynamics of specific antibodies in the serum of the patient.

**Interventions.** Them concern:

1) The operations connected with an infection of skin wounds, mucous membranes, and also with infectious complications on the operated body;

2) Injections of medical and preventive preparations in which result arise infiltration, an abscess, a phlegmone;

3) Blood transfusion and its substitutes, vessels, a hemodialysis, hemadsorption which can lead to a thrombosis of vessels, to an abscess of soft fabrics;

4) To a cystitis, a pyelonephritis;

5) Hardware artificial breath, washing of bronchial tubes, aerosol introduction of solutions of antiseptic tanks and antibiotics in which result can arise a bronchitis, a pneumonia, a laryngitis, a lung gangrene, a pleurisy and even a sepsis;

6) Stomatologic manipulations which can lead to development of a stomatitis, an abscess and a phlegmon of soft fabrics, to a jaw osteomyelitis, a sinusitis, a brain abscess;

7) Abortions, researches in the field of sexual sphere, can be the occurrence reason suppuration.

Frequency of hospital infections, structure of activators, forms of hospital infections, localisation, risk factors of hospital infections appreciably depend on type of hospital branch, but in all cases joining hospital infections to the basic disease
worsens illness outcomes, including raises lethality and frequency of transition of illness in the chronic form, increases duration of stay of the person in a hospital, expenses on leaving and treatment, labour losses.

**Immunity.**

At normal functioning of factors of nonspecific protection of an organism yatrogenical infections develop seldom even in case of penetration of activators into the internal environment of an organism through the damaged covers.

Decrease in local protection and the general nonspecific resistance sharply raises risk of development of an infectious disease.

Ability of immune system to development of the immune answer to antigens of is conditional-pathogenic bacteria - activators yatrogenical infections at healthy people is developed to a lesser degree, than on antigens of pathogenic microorganisms.

Nevertheless at the normal status of immune system yatrogenical infections develop seldom.

The high dose of the activator and development of an immunodeficiency are necessary for their occurrence during yatrogenical for an infection which can lead to process generalisation, to its transition in the chronic form.

In case of occurrence of an infectious disease (complication) during stay of the patient in a hospital or after polyclinic visiting, and also after medical interventions it is necessary to establish yatrogenical the disease nature.

**Infection consider** yatrogenical if disease has arisen after polyclinic visiting where the patient was exposed to medical interventions through a time interval of not less minimum incubatory period of illness.
For opportunistic infections this term is equal to 2-4 days, for the infections caused by pathogenic microorganisms, it is distinguished and defined by character of an infectious disease.

More reliable data about yatrogenicity the arisen disease gives *microbiological research.*

Its principles same, as at an establishment of the activator of any infectious disease.

However in this case to research is exposed not only the patient, but also medical workers and other prospective sources of an infection, including objects of environment which could serve as factors of transfer of the activator.

Allocation from the sick sick-list ecological variants, even without an establishment of a source and ways of transfer of the activator, is the sufficient basis for reference of the given infectious disease to yatrogenical.

The microbiological control over hospital infections is an obligatory part of supervision of treatment-and-prophylactic establishments, first of all behind hospital hospitals.

It includes research of patients and the medical personnel on bacteriacarrier, objects of environment and medical products for the purpose of their establishment microbic kontamination first of all sick-lists ecological variants.

**Diseases of the infectious nature** which arise in hospitals (hospital infections).

In infectious hospitals observance sanitary-epidemiologic a mode provides distribution of patients on nosological forms in box, current and final disinfection, the microbiological control.
Aetiology: basic activators hospital an infection is conditional-pathogenic microbes.

The reasons:
1) objective, not dependent on the medical personnel;
2) the subjective.

1. Objective:
Hospitals, the branches mismatching requirements;
Absence of effective methods of treatment staphylococcal carrier and conditions for hospitalisation;
Insufficient number of bacteriological laboratories;
Unfairly wide application of antibiotics;
Set antibiotic resistant microorganisms;
Increase in persons with the lowered immunity.

2. Subjective:
Insufficient prophylactic the directed activity of the medical personnel;
absence of the uniform epidemiological approach to studying hospital an infections;
Absence of the due control from workers of the centres of Gossanepidnadzors;
Absence of reliable sterilisation of some kinds of equipment;
Increase in number of contacts between patients;
Absence of full account hospital an infections;
Poor quality of sterilisation of medical toolkit and disinfection;
Imperfect system of visitings by relatives.

Epidemiology:
1. Infection Sources:
1) the medical personnel, the visitors, suffering infectious diseases (a flu, a diarrhoeia, pustulous);
2) patients with the erased forms;
3) patients with the pure wounds, being carriers virulens staphylococcal strains;
4) chest children with a pneumonia, an otitis, a flu, allocating pathogenic strains an intestinal stick.

II. Mechanisms of a transmission of infection: aerogenic (respiratory, air-drop), fekal-oral, contact, parenteral (hepatitis B, D, delta, HIV).

Contributing factors:
1) easing of the patient;
2) duration of stay in a hospital (70 % hospital infections at the patients laying more of 20 days);
3) excessive application antibiotics, they change биоценоз intestines, reduce immunological resistance;
4) hospitalisation of a considerable quantity of people of old age, chronic patients who are a source of intrahospital infections;
5) stay in a hospital of small children, especially till 1 year;
6) the big density of patients in a hospital.

For prevention of distribution of intrahospital infections it is necessary:
- To spend planned complex epidemiological and bacteriological inspection of hospitals as the current control of 4-5 times in a year;
- To spend microbiological inspection of the personnel, patients, toolkit, medicines, food. Researches to spend periodically and in concrete cases at suspicion on danger of development of an intrahospital infection of epidemiological and not epidemiological character;
- System of box, special ventilation and other modern measures matter only when the personnel strictly all requirements of a sanitary mode in a hospital. To avoid many infections, it is necessary to apply as much as possible materials only disposable using.

**Laboratory Diagnosis of the hospital infection**

There are five approaches to the diagnosis of viral diseases by the use of clinical specimens:

1. identification of the virus in cell culture,
2. microscopic identification directly in the specimen,
3. serologic procedures to detect a rise in antibody titer or the presence of IgM antibody,
4. detection of viral antigens in blood or body fluids,
5. detection of viral nucleic acids in blood or the patient's cells.

**IDENTIFICATION IN CELL CULTURE**

The growth of viruses requires cell cultures, because viruses replicate only in living cells, not on cell-free media the way most bacteria can. Because many viruses are inactivated at room temperature, it is important to inoculate the specimen into the cell culture as soon as possible; brief transport or storage at 4 °C is acceptable.

Virus growth in cell culture frequently produces a characteristic cytopathic effect (CPE) that can provide a presumptive identification.

The time taken for the CPE to appear and the type of cell in which the virus produces the CPE are important clues in the presumptive identification.

If the virus does not produce a CPE, its presence can be detected by several other techniques:
(1) **Hemadsorption**, ie, attachment of erythrocytes to the surface of virus-infected cells. This technique is limited to viruses with a hemagglutinin protein on their envelope, such as mumps, parainfluenza, and influenza viruses.

(2) **Interference** with the formation of a CPE by a second virus. For example, rubella virus, which does not cause a CPE, can be detected by interference with the formation of a CPE by certain enteroviruses such as echovirus or coxsackievirus.

(3) A decrease in acid production by infected, dying cells. This can be detected visually by a color change in the phenol red (a pH indicator) in the culture medium.

The indicator remains red (alkaline) in the presence of virus-infected cells but turns yellow in the presence of metabolizing normal cells as a result of the acid produced. This technique can be used to detect certain enteroviruses.

**A definitive identification** of the virus grown in cell culture is made by using known antibody in one of several tests. Complement fixation, hemagglutination inhibition, and neutralization of the CPE are the most frequently used tests.

Other procedures such as fluorescent antibody, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and immunoelectron microscopy are also used in special instances. A brief description of these tests follows. They are described in more detail in the section on immunology.

**Complement Fixation.** If the antigen (the unknown virus in the culture fluid) and the known antibody are homologous, complement will be fixed (bound) to the antigen-antibody complex.

This makes it unavailable to lyse the "indicator" system, which is composed of sensitized red blood cells.
**Hemagglutination Inhibition.** If the virus and antibody are homologous, the virus is blocked from attaching to the erythrocytes and no hemagglutination occurs. Only viruses that agglutinate red blood cells can be identified by this method.

**Neutralization.** If the virus and antibody are homologous, the antibody bound to the surface of the virus blocks its entry into the cell.

This neutralizes viral infectivity, because it prevents viral replication and subsequent CPE formation or animal infection.

**Fluorescent-Antibody Assay.** If the virus-infected cells and the fluorescein-tagged antibody are homologous, the typical apple-green color of fluorescein is seen in the cells by ultraviolet (UV) microscopy.

**Radioimmunoassay.** If the virus and the antibody are homologous, there is less antibody remaining to bind to the known radiolabeled virus.

**Enzyme-Linked Immunosorbent Assay (ELISA).** First, the antibody is bound to a surface. If the virus is homologous, it will be bound also.

A sample of the antibody linked to an enzyme is added, and the amount of enzyme is assayed.

**Immunoelectron microscopy.**

If the antibody is homologous to the virus, aggregates of virus-antibody complexes are seen in the electron microscope.
MICROSCOPIC IDENTIFICATION

Viruses can be detected and identified by direct microscopic examination of clinical specimens such as biopsy material or skin lesions.

Three different procedures can be used.

1. Light microscopy can reveal characteristic inclusion bodies or multinucleated giant cells. The Tzanck smear, which shows herpesvirus-induced multinucleated giant cells in vesicular skin lesions, is a good example.

2. UV microscopy is used for fluorescent-antibody staining of the virus in infected cells.

3. Electron microscopy detects virus particles, which can be characterized by their size and morphology.

SEROLOGIC PROCEDURES

In the third approach, a rise in the titer\(^1\) of antibody to the virus can be used to diagnose current infection.

A serum sample is obtained as soon as a viral etiology is suspected (acute-phase), and a second sample is obtained 10-14 days later (convalescent-phase).

If the antibody titer in the convalescent-phase serum sample is at least 4-fold higher than the titer in the acute-phase serum sample, the patient is considered to be infected.

For example, if the titer in the acute-phase serum sample is 1/4 and the titer in the convalescent-phase serum sample is 1/16 or greater, the patient has had a significant rise in antibody titer and has been recently infected.

If, however, the titer in the convalescent-phase serum sample is 1/8, this is not a significant rise and should not be interpreted as a sign of recent infection.
It is important to realize that an antibody titer on a single sample does not
distinguish between a previous infection and a current one. The antibody titer can be
determined by many of the immunologic tests mentioned above.

These serologic diagnoses are usually made retrospectively, because the disease
has frequently run its course by the time the results are obtained.

In certain viral diseases, the presence of IgM antibody is used to diagnose current
infection. For example, the presence of IgM antibody to core antigen indicates
infection by hepatitis B virus.

Other nonspecific serologic tests are available. For example, the heterophil
antibody test (Monospot) can be used to diagnose infectious mononucleosis.

**DETECTION OF VIRAL ANTIGENS**

Viral antigens can be detected in the patient's blood or body fluids by various
tests but most often by an ELISA. Tests for the p24 antigen of HIV and the surface
antigen of HBV are common examples of this approach.

**DETECTION OF VIRAL NUCLEIC ACIDS**

Viral nucleic acids, ie, either the viral genome or viral mRNA, can be detected in
the patient's blood or tissues with complementary DNA or RNA (cDNA or cRNA) as a
probe. If only small amounts of viral nucleic acids are present in the patient, the
polymerase chain reaction (PCR) can be used to amplify the viral nucleic acids.

Assays for the RNA of HIV in the patient's blood (viral load) are commonly used
to monitor the course of the disease and to evaluate the patient's prognosis.
Microbiologic investigations in non-infectious clinic.

Microbiologic investigations of the suppurative lesions are conduct – with the aim of diagnostic, researching of the etyologic factors and determination of the sensitivity to antibacterial remedies.

The results of the tests can help to select the most effective remedies for the treatment and also it can help to lead the prophylactic measures of hospital infections in the time.

It’s important to maintain some demands to the order during the getting of the material, its transport to the laboratory, methodic of testing and estimation of the result.

I. Microbiologic investigation of urine.

Microbiologic investigation of urine is conduct if there is the suppurative lesions of the urologic tract.

Urinary diseases are urethritis, cystitis, pyelonephritis, urosepsis. As a rule the infections agents of the urinary diseases are the Escherichia coli, proteus, klebsiella, pseudomonas, mycoplasma, enterococcus, staphylococcus, serratia, enterobacter, yeast like Candida fungus, etc.

The rules for the gathering of the material. It’s necessary to lead the researching before the begining of the antibacterial therapy. It’s gathered the middle portion of the urine (3-5ml) to the sterile dish.

It’s important to lead the researching as soon as it possible it must help to avoid the reproduction of the available in the urine microorganisms.

For determination of the bacteriuria degree is used the method of sector’s inoculation on nutrient agar.
Degree of the bacteriuria is a number of colony-forming units (CFU) per 1ml of urine.

**Research methodologies.**

1. Flame the loop. Take centrifuged deposit of urine and streak one loopful over Sector A near edge of the plate. Apply the loop lightly. Don’t gouge into the medium.

2. Flame the loop, cool 5 seconds, and make 4 streaks from Sector A through Sector 1. Momentarily touching the loop to a sterile area of the medium before streaking insures a cool loop.

3. Flame the loop again, cool it, and make 4 streaks from Sector 1 through Sector 2.

4. Flame the loop again, cool it, and make 4 streaks from Sector 2 into Sector 3, using up the remainder of the plate surface.

5. Flame the loop before putting it aside.
The inoculated media are incubated in termostat at 37ºC for 24-48 hours. After incubation, the number of colony-forming units on the sectors is calculated.

Determination of the bacteriuria degree make according to the table 1.

**Table 1. Determination of bacteriuria intensity**

<table>
<thead>
<tr>
<th></th>
<th>Number of bacteria in sectors</th>
<th>Number of bacteria per 1 ml of urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1-6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8-20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21-30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>31-60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>70-80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100-150</td>
<td>5-10</td>
<td>-</td>
</tr>
<tr>
<td>Don't calculate</td>
<td>20-30</td>
<td>-</td>
</tr>
<tr>
<td>Don't calculate</td>
<td>40-60</td>
<td>-</td>
</tr>
<tr>
<td>Don't calculate</td>
<td>100-140</td>
<td>10-20</td>
</tr>
<tr>
<td>Don't calculate</td>
<td>Don’t calculate</td>
<td>30-40</td>
</tr>
<tr>
<td>Don't calculate</td>
<td>Don’t calculate</td>
<td>60-80</td>
</tr>
</tbody>
</table>

Sub-inoculated of growing colony onto slant agar. Isolating pure cultures of bacteria are identified and then determined sensitivity to antibiotics.
**Estimation of results.**

Necessary take into account complex of tests:

# degree of the bacteriuria,
# species of the isolating pure cultures,
# frequency of isolation them during of the disease,
# them presence into urine in monoculture or associations.

Estimation of results according to the next criteria:

1. Degree of the bacteriuria is $10^3$ CFU/ml of urine. It is corresponds to absence of the inflammatory process and usually may be result of the urine contamination.

2. Degree of the bacteriuria is $10^4$ CFU/ml of urine. It is doubtful result. Bacteriologist will have to repeat this research.

3. Degree of the bacteriuria is $10^5$ CFU/ml of urine and higher. It is the inflammatory process.

For the complete estimation of the results it is necessary to account the results of another laboratory tests and facts from the clinic.

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**II. Microbiologic investigation of the sputum.**

It is convied in occurence of the inflammatory diseases of the respiratory system: pneumonia, bronchitis, pleuritis, the lung abscess etc.

The infectious agents of the lower respiratory tract may be pneumococci, staphylococci, pseudomonas, klebsiella, mycoplasma, rickettsia, fungi, viruses, protozoa etc.

Sputum is the most common specimen collected in suspected cases of the lower respiratory tract infections.
Sputum is the mucous secretion expectorated from the lungs, bronchi, and trachea through the mouth, in contrast to saliva, which is the secretion of the salivary glands that contains oral microflora.

The rules for the gathering of the material:
1) the gathering of the material is before the begining of the antibacterial therapy;
2) research the morning portion of the sputum;
3) before the gathering of the sputum the patient has to clean the teeth and wash his mouth by the water;
4) the sputum is collected in specially designed sputum cups;
5) the material is stored in refrigerator but no longer than 2-5 hours.

**Research methodologies.**

Prepare the 2 smears. One of them stained by Gram, another – by Ziehl-Neelsen (for the determination of the tuberculosis bacillus in the smear).

Streak the sputum to differential nutrient media:
# for the detection of pathogenic staphylococci on 5% blood agar; medium etc.
# for the detection of E.coli on Endo medium;
# for the detection of fungi on Sabourad medium;
# for the detection of anaerobic bacteria on Kitta-Tarrosi medium etc.

During the next 24 hours the media are stored at the termostat (37ºC).
Then the pure cultures of bacteria are isolated from the grown cultures, identified and determined sensitivity to antibiotics.
**Theme:** Microbiological investigation in non-infectious clinic. Features of taking material, delivery, analysis.

**The main aim of the lesson is:**
- to learn the main methods of laboratory diagnostics in clinical microbiology.

**Questions for preparing for practical classes.**
1. Role of conditionally pathogenic microbes in human pathology.
2. Main clinical manifestation of diseases caused by conditionally pathogenic microbes.
3. Biological properties of the main conditionally pathogenic microbes (microbes from genus Staphylococcus, Streptococcus, Enterococcus, Escherichia, Proteus, Pseudomonas, Klebsiella).

**Practical work of students.**
1. To study the main tasks of clinical microbiology.
2. To learn the main principles and rules of collection and culturing of specimens.
3. To study characteristics of conditionally pathogenic bacteria and diseases they are caused.
4. To study Etiology and methods of laboratory diagnosis of wound infections.
5. To study Etiology and methods of laboratory diagnosis of septic infections.
6. To study Etiology and methods of laboratory diagnosis of respiratory tract and mouth infections
7. To study Etiology and methods of laboratory diagnosis of urinary tract infections.
8. To study Etiology and methods of laboratory diagnosis of nervous system infections.


10. To learn Etiologic criteria of bacterial significans.

11. To solve situational tasks.

**Theme:** Hospital infection. Microbiological diagnostics of the hospital infection.

**Questions for the learning.**

1. Hospital infection.

2. Characteristic and biological properties of the causative agents of hospital infection (staphylococcus, E. coli, enterococcus, pseudomonas aeruginosa, proteus and other), diseases.

3. Role of the causative agents of the hospital infection.

4. Laboratory diagnostic of the hospital infection.

   **I. Name the term:**

   *Hospital infection*_____________________________________________________

   ____________________________________________________________

   ____________________________________________________________

   ____________________________________________________________

   2. The characteristic of activators of the hospital infection:

   #________________________________

   #________________________________

   #________________________________

   #________________________________
3. Deciphering of the etiology of the hospital infection:

4. Principal causes of development of the hospital infection.

5. Fill in the table. The basic nosological forms of the hospital infection.

<table>
<thead>
<tr>
<th>Generalized forms</th>
<th>The diseases of a skin and subcutaneous fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6. Fill in the table. **The basis agents of the hospital infection:**

<table>
<thead>
<tr>
<th>Acute clinical infectious</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary diseases</td>
<td></td>
</tr>
</tbody>
</table>

7. **Microbiological diagnostics of the hospital infection.**

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Theme: Microbiological investigations in non infectious clinic. Features of taking a material, delivery, analysis.

Questions for the learning.
1. Role of conditioned pathogenic microorganisms in pathology of non infectious diseases. (staphylococcus, Candida albicans, E. coli, proteus, klebsiella, enterococcus, pseudomonas aeruginosa and other).
2. Characteristic and biological properties of the conditioned pathogenic microorganisms.
3. Factors leading the appearance and development of the pathogenic processes.
4. Rules of taking of urine and sputum for the bacteriological research.
5. Main stages of the bacteriological researching.
6. The bacteriological analysis of the material taking from the patients with diseases of the respiratory organs and urogenital system.
7. Interpretation of the results.
   1. Name the aim of microbiological investigations in non infectious clinic.

2. Microbiological investigation of the urine.
# Enumerate microorganisms – infectious agents of the urogenital inflammatory diseases:

_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

_____________________________________________________________________

# Rules of taking of the urine for the bacteriological research.

_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

# Draw the scheme of the urine inoculation on the nutrient agar.

**Nutrient agar**
3. Microbiological investigation of the sputum.

# Enumerate microorganisms – infectious agents of the inflammatory diseases of the respiratory system:

# Rules of taking of the sputum for the bacteriological research.

# Main stages of the bacteriological researching of the sputum.
1. Using a quantitative method to define the content of a specimen of a certain
type of microorganisms. What are the critical number allows to judge about the
etiological role of microorganisms in disease?
A. $10^6 - 10^7$
B. $10^5 - 10^6$
C. $10^4 - 10^5$
D. $10^3 - 10^4$
E. $10^2 - 10^3$

2. In diseases of the upper respiratory tract in most cases the initial
microbiological method of research is on microscopy. This allows to identify
smear microscopy?
A. Capsulorrhesis forms of bacteria
B. Mobile forms of bacteria
C. Trudnosorbiruemye microorganisms
D. Spore-forming microorganisms
E. L-form bacteria

3. Microbiological examination of the respiratory system begin with microscopy
of smears prepared from sputum. The nature of any further research determines
the initial smear?
A. Allergic
B. Culture
C. Serology
D. Biological
E. Biochemical

4. When mikrobiologicheskim study of the urine of the patient urology
Department, the degree of bacteriuria was $10^3$. As can be seen the result?
A. Dubious Results, it is necessary to conduct additional planting.
B. Result indicates urinary tract infections
C. Urine contaminated with microorganisms from the environment
D. Urine contaminated natural microflora
E. Scarcity of growth is a consequence of antibiotic therapy

5. To obtain the most accurate results of microbiological studies, or to comply
with the rules of transportation and storage of native clinical sample. What
causes prolonged and improper storage of clinical material?
A. Death of microorganisms due to the lack of nutrients
B. Contamination of the material by other organisms.
C. Violation of the quantitative relationships of species of microorganisms
D. Change of morphological properties of microorganisms
E. Changing cultural properties of microorganisms

6. What are the microorganisms most often cause purulent oslojneniya surgical
wounds?
A. Proteus
B. E. coli
C. Klebsiella
D. Staphylococci
E. Streptococci

7. What are the consequences of violations of the rules of strict asepsis during the
capture of native material for microbiological research?
A. Contamination of the sample by the microflora of the environment
B. Contamination environmental microflora samples
C. the Emergence of nosocomial infections
D. Infection med staff
E. Emergence of nosocomial strains of drug-resistant