МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
Харківський національний медичний університет

CAUSATIVE AGENTS OF OPPORTUNISTIC INFECTIONS

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology)

ЗБУДНИКИ ОПОРТУНІСТИЧНИХ ІНФЕКЦІЙ

Методичні вказівки з дисципліни "Мікробіологія, вірусологія та імунологія" для студентів II і III курсів медичного та стоматологічного факультетів з англійською мовою викладання

Затверджено
вченою радою ХНМУ.
Протокол № 4 від 19.04.2018.

Харків
ХНМУ
2018

Causative agents of opportunistic infections: Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology) / N. I. Kovalenko, T. M. Zamaziy. – Kharkiv : Kharkiv National Medical University, 2018. – 36 p.

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Збудники опортуністичних інфекцій : метод. вказ. з дисципліни "Мікробіологія, вірусологія та імунологія" для студентів II і III курсів мед. та стомат. фак-тів з англ. мов. викладання / упоряд. Н. І. Коваленко, Т. М. Замазій. – Харків : ХНМУ, 2018. – 36 с.

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Theme: LABORATORY DIAGNOSIS OF OPPORTUNISTIC INFECTIONS

Actuality of the theme.

Development of microbiology and teaching about infectious diseases led to the idea that certain pathogens are infectious agents with their characteristic clinical manifestations, routes of transmission and features distribution (epidemiology). However, diseases caused by microorganisms, usually accompanying persons and harmless to them have spread since the second half of the XX century. These are E. coli that permanently resides in the intestines, staphylococcus – saprophytes of skin, haemophilic microflora of the upper respiratory tract and others. These microorganisms have been named opportunistic, because their pathogenic action is manifested only under certain conditions: the weakening of the host organism or the acquisition of special properties by the causative agent (resistance to antibiotics, the ability to produce toxic substances, etc.). It is established that excessive colonization of an organism by any type of bacteria capable of surviving in the human body, can lead to the development of pathology.

The range of opportunistic microorganisms is very broad. These include many representatives of the family of intestinal bacteria (Klebsiella, Proteus, Providencia, Serracia, Citrobacter, Enterbacter), cocci (staphylococci, streptococci of group B, enterococci), Pseudomonas aeruginosa, asporogenic anaerobes, and others.

Opportunistic representatives of the Enterobacteriacea family can cause various clinical syndromes, inflammatory processes of different localization and sepsis. Of all clinically significant bacteria enterobacteria make up about 50 %, with Gram-negative - about 80 %. The part of enterobacteria accounts for about 50 % of all pathogens of septicemia, up to 70 % of gastroenteritis and more than 70 % of pathogens of urinary tract infections. If we take into account the immunodeficiencies and the spread of hospital infections, it can be said that enterobacteria can be isolated from any material delivered to the laboratory.

Since a number of infections often endanger the life of the patient, there is a need for rapid isolation, identification of relevant pathogens and detection their sensitivity to antimicrobial drugs.

Goal: Studying of laboratory diagnostics of Klebsiella, Proteus and P.aeruginosa infections.

Concrete goals:

1. Study of biological properties and classification of Klebsiella, Proteus and P. aeruginosa.
2. Study pathogenesis and clinical manifestations of Klebsiella, Proteus and P. aeruginosa infections.
3. Study of the methods of laboratory diagnosis of Klebsiella, Proteus and P. aeruginosa infections.

Students should be able to:

1. Isolate of pure cultures of Klebsiella, Proteus and P. aeruginosa and examine growth on differential media.
2. Identify of pure culture of Klebsiella, Proteus and P. aeruginosa on morphology, culture and biochemical properties, antigenic structure.
3. Interprete results of serological tests to diagnose Klebsiella, Proteus and P.aeruginosa infections.

Equipment: slides, immersion microscope, biological preparations for laboratory diagnosis of Klebsiella, Proteus and P. aeruginosa infections, tables, atlas.

Klebsiella

The genus Klebsiella, named after E. Klebs, includes 4 species, the first of which Klebsiella pneumoniae consists of three subspecies: K. subsp. pneumoniae, K. ozaenae and K. rhinoscleromatis.

Taxonomy.

Family: Enterobacteriaceae

Genus: Klebsiella

Species: K. pneumoniae, K.oxytoca, K.planticola, K.terrigena

The Klebsiella genus combines capsular bacteria that cause various diseases:

– bronchopneumonia, meningitis, appendicitis, cystitis and purulent-inflammatory processes, but more often intestinal infections, affecting the lower parts of the large intestine – K. pneumoniae pneumoniae;

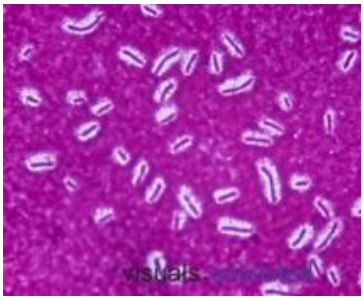
– rhinoscleroma – K. pneumoniae rhinoscleromatis, chronic granulomatous process on the skin, nasal mucosa, trachea, larynx, bronchi;

– ozena (fetid runny nose) – lesions of the nasal mucosa, pharynx, trachea, larynx, additional nasal cavity and nasal congestion – the secretion of a viscous secretion that dries with the formation of dense crust, which makes breathing difficult and has a fetid odor – K. pneumoniae ozaenae.

Klebsiella spp. are found in the mucus from the pharynx and the nose as a representative of normal microflora, secretions from the respiratory tract and lungs, on the objects of the environment.

Morphology. Klebsiella spp. are Gram-negative short thick rods, in size 0,6–6,0×0,3–1,5 microns with rounded ends, nonmotile, do not produce spores, form a capsule (*Fig. 1A*). In smears they are located singly, in pairs or short chains.

Cultural properties. Facultative anaerobes. They grow on simple nutrient media at 35–37 °C, pH 7.2. On solid media Klebsiella spp. form large dome-shaped mucous colonies (*Fig. 1B*), in the broth – intense turbidity. They are easily cultivated on Endo, EMC, Ploskirev. On Endo agar colonies are brightly colored with metallic shine.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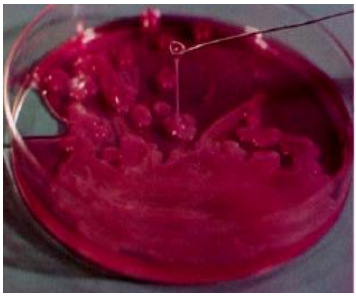
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Fig. 1. Klebsiella pneumoniae: A - morphology (Burry-Gins stain);
B – slimy mucoid, raised growth of Klebsiella which strings into threads when drawn with a loop

Enzymatic properties. Klebsiella spp. are biochemicaly active. They ferment lactose, break down glucose and mannitol with the formation of acid and gas, decompose urea, do not produce indole and hydrogen sulfide. Oxidase-negative and catalase-positive. The differentiation of Klebsiella subspecies is carried out on biochemical activity (*table 1*).

Table 1

Differentiation of Klebsiella subspecies on biochemical activity

Species	Properties				
	fermentation		utilization		Indol production
	glucose	lactose	urease	malonate	
K. subsp. pneumoniae	AG	A	+	+	-
K. subsp. ozaenae	v	A	v	-	-
K. subsp. rhinoscleromatis	AG	-	-	+	-

Designations: AG – up to acid and gas, A – to acid, v – propertie is variable, + – presence of characteristic, - – no sign.

Antigenic properties. Klebsiella spp. contain capsular K- and somatic O-antigens. The combination of these antigens determines the belonging of the cultures to the serovars. Currently, 80 K- and 11 O-antigens are known.

Resistance. Due to the presence of capsules, Klebsiella spp. are stable and persistent in the soil, water, on objects of consumption, vegetables, fruits, on the skin and mucous membranes of humans and animals. They die at 65 °C for an hour, sensitive to the action of solutions of disinfectants (chloramine, phenol, etc.). High resistance to antibiotics is noted. In dairy products, they survive and multiply when stored in the refrigerator and at room temperature.

Pathogenicity factors. Capsule, fimbria, thermostable and thermolabile enterotoxins; in some virulent strains there are enzymes of pathogenicity such as DNAse, neuraminidase, phosphatase.

Epidemiology. K. pneumoniae is part of the facultative microflora of the intestine, upper respiratory tract, vagina, also it is found on the skin and mucous membranes. K. pneumoniae is isolated from the oropharynx and gastrointestinal tract in 5 % of healthy people.

Under natural conditions, diseases of different animals are caused: cows, pigs, horses (mastitis, pneumonia, septicemia).

When exogenous infection the source of infection is a sick person and a healthy carrier.

Routes of transmission: fecal-oral, air-drop, household contact (dirty hands, household items). In children's institutions and hospitals, infection is often transmitted through linen, tools, toys. Factors of transmission: meat and dairy foods, water, air.

Pathogenesis of human diseases. ***Klebsiella pneumoniae*** was initially considered the causative agent of respiratory diseases (rhinoscleroma, ozena, pneumonia). Then it was found that they can cause damage to the mucous membranes of the eyelids, urogenital organs, as well as the meninges, joints, they are capable of causing sepsis, acute intestinal infections in adults and children, purulent postpartum complications. Infection of newborns can lead to the development of severe pneumonia, intestinal infections, toxic-septic conditions with a fatal outcome. Klebsiella are considered the causative agents of nosocomial infections. They are common in mixed infections.

Klebsiella ozena is the etiological factor of chronic diseases of the respiratory system. It affects the pharynx, trachea, larynx. The inflammatory process is accompanied by the release of a viscous secret that produces a fetid odor.

Klebsiella rhinoscleromatis causes chronic inflammation of the mucous membranes of the upper respiratory tract, bronchi with the formation of infiltrates, which then scar. Pathogens can be found in granulomas, where they are localized inside and outside of the cells.

Immunity. The disease causes the accumulation of antibodies to the Klebsiella, but they do not have significant protective properties. In the recovery, the main role belongs to phagocytosis, which is activated by specific opsonins. The emergence of chronic forms of Klebsiella infections is facilitated by intracellular localization of the pathogen and the development of the reaction of delayed hypersensitivity.

Laboratory diagnostics. Material for examination: sputum, mucus from the ulcer, pus from the ear, wound exudate, blood, urine, feces, rinsing from the objects of the environment.

Laboratory diagnosis of Klebsiella infections is based on the isolation of the pure culture of the pathogen, its differentiation from other enterobacteria and identification of the species according to morphological, cultural and biochemical characteristics, and the definition of the serovar. The serological method is the CFT with O-antigen of Klebsiella on the 7–8 day of illness with suspected rhinoscleral disease.

Scheme of laboratory diagnosis of Klebsiella infections

Specimen: sputum, wound exudates, urine.

1. Microscopy: Gram stain. Klebsiella spp. appear as short, plump, Gram-negative bacilli. Burry-Gins stain. They are usually surrounded by a capsule that appears as a clear space.

Specimen: nasal swab, sputum, wound exudates, blood, urine, pleural fluid, peripheral or central intravenous access sites, urinary catheters, respiratory support equipment.

2. Culture: Klebsiella spp. grow well on most ordinary media.

Identification:

– Biochemical tests:
  - lactose-positive,
  - urease-positive,
  - methyl red-positive,
  - indole-negative (K. oxytoca and some strains of K. pneumoniae are exceptions).
  - H_2S-negative.

– Slide AT and IF with type-specific capsular sera.

– Phagetyping.

3. Serology (scleroma): CFT, PHAT (detection of antibodies).

Prevention and treatment. Vaccine prophylaxis of Klebsiella infections has not been developed. Specific prophylaxis – the use of polyvalent purified liquid bacteriophage.

For treatment, β-lactams, aminoglycosides, tetracyclines are used. Over the past few years, antibiotic-resistant strains of Klebsiella have been widely spread.

Laboratory algorithms:

Alorithm: *"The course of microbiological analysis of a material for suspected Klebsiella infection."*

The purpose of the analysis is isolation and identification of Klebsiella spp. from pathological material and objects of the environment.

Day 1: seeding of pathogenic material on MPA, Endo and Ploskirev media, sugar broth. Incubation at 37 °C for 24 hours.

Day 2: preparation of smears and microscopy (Gram stain) from mucous colonies (on Endo agar – pink, white or red, on Ploskirev – beige or yellow). In the presence of Gram-negative rods 4-5 colonies are selected, trasfered to slant agar and combined Ressel medium to study the enzymatic properties and motility of the isolated pure culture. In a test tube under the cap, strips of paper imprinted with reagents are placed to determine the formation of indole and hydrogen sulfide. Seeding on Preuss medium with urea, on Simmons citrate agar. Incubation at 37 °C.

Day 3: accounting for results of growth. With the growth of a nonmotile culture that:

– fermentes lactose, glucose, urea,

– does not form indole and hydrogen sulfide,

– hydrolyzes or does not hydrolyze urea,

– utilizes citrate as the only source of carbon, smears are prepared to determine a capsule after Burry stain. In the presence of capsule, AT is performed on a slide with K-sera to differentiate K. ozaenae and K.rhinoscleromatis and O-agglutination. With a positive result of capsular agglutination, agglutinate is formed in the form of coarse threads or strands. For O-agglutination reaction, the culture is autoclaved for 2.5 hours. As a result, bacteria lose the capsule and the ability to interact with K-sera, but acquire agglutinabeliness with O-sera. After sterilization, the culture is washed 2 times by centrifugation and slide AT is performed.

Serologic method: CFT for 7–8 days of the disease in case of suspicion of rhinosclerotic disease (with patient's serum in dilution 1 : 100–1 : 1 600 and a scleromatic diagnosticum of killed Klebsiella). The increase in the antibody titre in the dynamics of the disease is a confirmation of the diagnosis.

Proteus

Taxonomy.

Family: Enterobacteriaceae

Genus: Proteus

Species: Proteus vulgaris, Proteus mirabilis.

The Proteus genus combines several species that differ in the ability to ferment a lot of nutritional substrates. Morphologically, they do not differ from E. coli. Bacteria are widespread in the environment. They are found in soil, air, water, large intestines of humans and animals, vagina of women. They relate to putrefactive opportunistic bacteria. Proteus spp. can cause various purulent diseases in human: suppuration of wounds, inflammation of the middle ear, peritonitis, pyelonephritis, cystitis, food poisoning, sepsis, and others.

Proteus genus includes three species; Proteus vulgaris and Proteus mirabilis play a role in the human pathology as causative agents of food toxic infections and purulent-inflammatory processes of different localization.

Fig. 2. Swarming growth of P. vulgaris

Morphology. Proteus spp. are motile (peritrichous) rods in the sizes 0.4–0.6 × 1.0–3.0 microns. There are coccoid and filamentous forms. They do not produce spores and capsules.

Cultural properties. Proteus spp. are facultative anaerobes, grow well on simple nutrient media at 20–37 °C. H-forms of bacteria give "swarming growth" on a solid nutrient medium (*Fig. 2*), and

when seeded in condensed water of the sloped agar – growth along the entire surface of the medium (the method of isolating pure culture by Shukevich) with the formation of daughter processes. On mediun with the addition of bile they form O-forms – large colonies with entire edges. On the broth they form cloudy, thick white precipitate and a gentle film on the surface. On solid media they produce intermidiate translucent colonies. Some strains cause hemolysis of erythrocytes in blood agar.

Enzymatic properties. They break down glucose, maltose, sucrose, and others carbohydrates with the formation of acid and gas, do not ferment lactose, mannitol. Proteolytic activity: they dilute gelatin, break down urea, form hydrogen sulfide, indole, ammonia. Oxidase-negative and catalase-positive.

Antigenic properties. Proteus spp. contain O- and H-antigens. There are 49 O-antigens, about 19 H-antigens and over 100 serotypes. The combination of O- and H-antigens in a microbial cell determines the identity of pathogens to O-serogroup or serovar.

Resistance. They are relatively stable in the environment and may be stored in the soil, sewage, in food products. Proteus spp. survive at 60 °C for an hour and are resistant to solutions of disinfectants, many antibiotics, and low temperatures.

Pathogenicity factors: flagella, fimbria, endotoxin, hemolysins, hemagglutinins, urease, proteases.

Epidemiology. Man is the source of infection. Proteus spp. usually enter into the environment with human defecation. Routes of transmission: food, household contact (dirty hands, linen, articles, surgical instruments).

Pathogenicity and the pathogenesis of human diseases. Proteus spp. are related to opportunistic bacteria. Their virulent properties are determined by the components of the cell wall, and toxic properties are associated with LPS, which are released after the destruction of microbial cells. When large doses of Proteus spp. enter the digestive tract, food-borne toxicity occurs, manifested as acute gastroenteritis. When bacteria penetrate in the body through the wound and burn area purulent-inflammatory processes develop in different organs.

P. mirabilis more often than P. vulgaris, causes purulent-inflammatory diseases and, first of all, the urinary system. These diseases can be a consequence of infection (transmission with a catheter, other urological tools), and sometimes they develop as an autoinfection with immunodeficiency states. Especially dangerous are infections of newborns - ingestion of Proteus spp. into the umbilical wound leads to bacteremia or the development of meningitis.

In the pathogenesis of urinary tract infections caused by Proteus spp., an important role is played by the urease, which splits urea, causes the release of ammonia, which leads to an increase in pH. Alkalization of urine reduces the solubility of calcium and magnesium, creating favorable conditions for the deposition of calcium and magnesium salts and the formation of kidney stones.

Microbiological diagnostics. Material for research: feces, vomit masses, urine, blood, mucous, pus from the ear, sectional material, swab from the environment.

Methods of research: Microbiological and serological.

The isolated pure cultures are identified by their cultural and biochemical properties.

Scheme of laboratory diagnosis of Proteus spp. infections.

Specimen: pus, wound exudates, conjunctival exudates, nasopharyngeal aspirate, urine, feces.

Culture on blood agar (swarming effect), on MacConkey agar (fishy odor, non-lactose fermenting colonies), on triple sugar iron agar (red with H_2S production).

Identification:

– Biochemical tests:
- lactose-negative,
- catalase- and nitrate-positive,
- methyl red-positive,
- urease-positive,
- H_2S-positive,
- oxidase-negative,
- Voges-Proskauer – variable.

Differentiation of P. mirabilis and P. vulgaris:
- Indole test: P. mirabilis (negative) and P. vulgaris (positive).
- Citrate test: P. mirabilis (positive) and P. vulgaris (negative).
- Slide agglutination test with polyvalent O-sera and type-specific H-sera.

– Phagetyping.

Immunity. Protective immunity is not formed.

Prevention. Specific prophylaxis – coli-proteus bacteriophage, intestine-bacteriophage (prophylaxis of enterocolitis in children's centers), piobac-teriophage (prophylaxis of hospital infections – for the treatment of fresh wounds).

Treatment. Gentamicin, carbenicillin, cephalosporins of the third generation, fluoroquinolones, and others. Nitrofurans (furagin, 5-NOC, and others) are used for treatment of the genitourinary organs.

Laboratory algorithms:

Algorithm: *"The course of microbiological analysis of a material for suspected Proteus infection"*.

Purpose of the analysis: isolation and identification of Proteus spp. from pathological material and objects of the environment.

Day 1: seeding pathogenic material on Ploskirev medium, enrichment medium – 5 % bile broth. Incubation at 37 °C for 24 hours;

Day 2: accounting for results of growth. On Ploskirev medium – transparent large isolated colonies with a pinkish-yellowish tint, around the colonies of a

yellowish halo due to alkalinization of the medium. Grown colonies are seeded on combined Ressel medium (with urea), in a condensate water in a test tube with a molten agar after Shukevich, urea-based medium after Preuss.

Day 3: accounting for results of growth, preparation of a microslide, Gram stain (Gram-negative small rods). Proteus spp. do not ferment lactose, fermente glucose with the formation of gas, mainly hydrolyze urea. In the sample on Shukevich agar, growth across the surface of the slant agar. Culture is seeded on additional Hiss media, mannitol broth (the formation of indole and hydrogen sulfide), semisolid agar, gelatin, on medium with amino acid phenylalanine (slant agar).

Day 4: accounting for results of growth: Proteus spp. do not ferment mannitol, form indole and hydrogen sulfide, motile, dilute gelatin and form the enzyme of phenylalanine desaminase (the appearance of dark green color when added to the surface of growth 10 % aqueous solution of $FeCl_3$). The most important feature that distinguishes Proteus spp. from other enterobacteria is the ability to desaminate phenylalanine. Strains attributed to biochemical properties to P.vulgaris or P. mirabilis are serologically typed in slide AT with polyvalent O-sera, then with typical and H-sera.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a typical species of the genus Pseudomonas - was first described by A. Lyuke in 1862. It refers to opportunistic microorganisms. At present, the incidence of inflammatory processes, in which the Pseudomonas aeruginosa is registered, has increased significantly.

Taxonomy.

Family: Pseudomonadoceae

Genus: Pseudomonas

Species: Pseudomonas aeruginosa

These bacteria are typical opportunistic microorganisms. They are widespread in the environment, constantly inhabiting the human body (5 % among healthy people) and animals, and are released from the soil, water. In healthy people, they are detected on the skin of the inguinal, armpit areas and ears (up to 2 %), nasal mucosa (up to 3 %) and pharynx (up to 7 %), in the gastrointestinal tract (3-24%).

Morphological and tinctorial properties. Pseudomonas aeruginosa are small Gram-negative rods with rounded ends. The average size is 1.5–3.0×0.5–0.8 microns. Motile, mono- or lophotrichous (has 1–2 polar flagella). In smears of pure cultures, rods are arranged singly, in pairs or in the form of short chains. In smears from pathological material, they can often be detected in the cytoplasm of phagocytes. They do not produce spores. Sometimes they form capsule-shaped extracellular mucus.

Cultural properties. Pseudomonas aeruginosa is aerobe but can grow in anaerobic conditions. It grows well on simple nutrient media. The optimum temperature of growth is 37 °C, but it can grow at 5–42 °C. On MPA, colonies of size 2–5 mm are formed, round, S-shaped, translucent, blue-gray with pearlescent shade (*Fig. 3A*), soldered with the medium, but it can form flat, irregularly shaped colonies with wavy edges or folded colonies with a rough surface ("daisies").

On solid media, many strains of Pseudomonas aeruginosa give the phenomenon of rainbow lysis (due to the spontaneous action of bacteriophages) – the appearance of a film on the surface of colonies, a rainbow, flowing in all colors. This phenomenon can be regarded as an additional taxonomic sign. On MPB they give turbidity, a precipitate and form a characteristic gray-silver film, as the culture ages there is turbidity of the medium in a direction from top to bottom.

A distinctive feature of a microorganism is the limited need for nutrients, which ensures the preservation of viability in conditions of almost complete lack of power supplies. Formation of mucus is a characteristic feature of virulent strains, mucus adds viscosity to broth cultures and colonies.

Characteristic feature of P.aeruginosa is pigment and aroma formation. Most strains form a blue-green pigment – piocyanin, which paints the nutrient medium (*Fig. 3B*), the wound exudate and dressing material. Pyocyanin is soluble in water. It has antagonistic properties for many bacteria, but it is toxic and therefore is not used for therapeutic purposes.

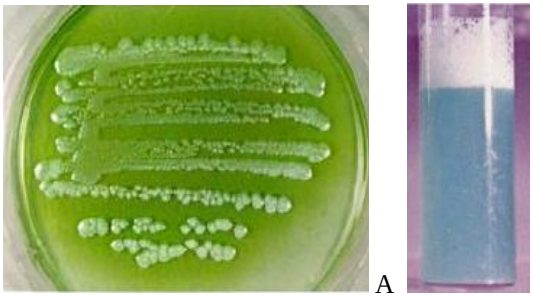

A B

Fig. 3. A – colonies of P. aeruginosa; B – piocyanin production

The formation of a pigment is an important diagnostic feature, which is observed in 70-80 % of clinical isolates. The vast majority of cultures also form the green pigment fluorescein (pioverdin), fluoresceive under UV light. Some strains can also synthesize other pigments: piroubin (red), piumelanin (black-brown) and L-oxyphenazine (yellow). The ability to synthesize and sensitivity to piocines vary widely in different strains, which is the base for the pyocinotyping of pseudomonads. It is commonly used in the epidemiological

evaluation of isolated cultures. Almost all strains of P.aeruginosa have a characteristic odor of jasmine due to the formation of trimethylamine.

Enzymatic properties. P. aeruginosa ferments only one carbohydrate – glucose to acid. Proteolytic activity is well expressed: it dilutes gelatin and coagulates the serum of blood, coagulates milk, destroys hemoglobin (β-hemolysis). It gives a positive reaction to cytochrome oxidase, which is one of the leading tests in identifying bacteria. Catalase-positive.

Antigenic properties. P. aeruginosa possesses O- and H-antigens. There is antigenic community with other pseudomonas. The mucous agent, toxins, adhesin, fimbria, and enzymes have antigenic properties and actively participate in the pathogenesis of P. aeruginosa infections. The belonging of the isolated culture to a specific O-serogroup is established in the agglutination reaction with O-sera, the type is determined by H-antigen. There are 17 serovars on the O-antigen.

Resistance. P. aeruginosa is stable in the environment. It dies at 60 °C for 15 minutes. 2 % solution of phenol destroys the P. aeruginosa. It is sensitive to 3 % solution of hydrogen peroxide, 2 % solution of carbolic acid, 0.1 % solution of sulfochloratin. It is stored in burn scales and dust (up to 2 weeks), in solutions used in medical practice during the year, on medical equipment. It is resistant to most antibiotics.

Factors of pathogenicity. P. aeruginosa forms exotoxin, which has hemolytic and cytotoxic activity:

– exotoxin A – its effect is manifested in the systemic toxic effect, edema, necrosis, and others;

– exoenzyme S causes pathological process in the lungs;

– cytotoxin exhibits a strong cytotoxic effect, including segmented neutrophils, contributing to the development of neutropenia, increases the permeability of cell membranes;

– two hemolytic substances: thermolabile hemolysin with lecithinase activity and thermostable hemolysin, causing necrotic lesions (especially in the liver and lungs);

– endotoxin, which leads to the development of pyrogenic reaction and stimulates inflammation;

– enterotoxin.

Factors of pathogenicity include enzymes of aggression: collagenase, protease, hyaluronidase, lecithinase, neuraminidase; factor of tissue permeability involved in damage; extracellular mucus can cause leukopenia, and has an antiphagocytic activity; adhesins, fimbriae.

Pathogenicity. P. aeruginosa has pili, responsible for the adhesion of microorganisms on the cells of the body; produces a number of toxins and enzymes, among which exotoxin A is the main, leading pathogenicity factor, which has a cytotoxic effect, hemolysins, leukocidin, as well as enzymes –

coagulase, elastase, etc. Pathogenicity of P. aeruginosa is based on mucus capsule-like substance that protects the microorganism against phagocytosis. Lipopolysaccharides of P. aeruginosa cells possess pyrogenicity and other properties characteristic of endotoxins.

Ecology and distribution. P. aeruginosa lives in soil, water, on plants. A wide spread contributes to easy infection of a person. Releasing from the patients, microorganisms persist for a long time on household items, medical instruments, especially – in wound exudate.

In hospitals there are strains of P. aeruginosa, usually highly resistant to antibiotics and antiseptics. Such strains can contaminate medications, remaining viable in antiseptics, disinfectant solutions.

Epidemiology. Sources of infection: a person (a patient with acute or chronic form of the disease and a carrier). Routes of transmission: mainly indirect contact, as well as parenteral (vascular catheters, solutions, blood products). Route of ransmission by air and dust has an important value. Endo- and exogenous infection.

Pathogenesis of human diseases. P. aeruginosa causes purulent-inflammatory processes in various tissues and organs. It is present in mixed infections. Pseudomonas infection occurs more often in children, elderly people and people with a decreased immune status. A purulent-inflammatory process develops with infection of wounds, urinary tracts and especially often - burn surfaces. P. aeruginosa is one of the main pathogens of nosocomial infection.

Diseases usually develop in people with reduced resistance: pneumonia (up to 20 %), cholecystitis, cystitis, pyelonephritis, otitis, inflammatory processes of the genitourinary system (one third of all lesions in urological patients), purulent-inflammatory complications after surgery (20–25 % among Gram-negative bacteria) and burns, sepsis, and others.

Bacteria are isolated in the intestine of 5 % of healthy people and up to 30 % of hospitalized patients.

Immunity is not strong.

Microbiological diagnostics. Material for diagnosis: mucus from the nose and pharynx, wound exudate, pus, blood, urine, sputum, secretion of the vagina or cervix, sectional material, rinsings from the objects of the environment and personnel hands.

Methods of study: bacteriological and microscopic:

Laboratory diagnosis is based on the isolation of the pathogen from pathological materials and the study of its biological properties. The main differentiating features: the reduction of nitrite to gaseous nitrogen, the dilution of 15 % of gelatin, the oxidation of glucose.

Intraspecific differentiation is carried out by phages, piocins, sensitivity to antibiotics and antiseptics.

Scheme of laboratory diagnosis of P. aeruginosa infections.

Specimen: sputum, pus, wound exudates, conjunctival exudates, urine, feces, blood, liquor.

1. Culture: the ability to grow at 42 °C distinguishes it from many other Pseudomonas species.

Identification:

– Biochemical tests:
  - lactose-negative,
  - oxidase-positive,
  - catalase-positive,
  - liquefaction of gelatin.

– Green pigment production on MPA with chicken embryo albumen.

– Fruity odor.

– Serotyping, pyocyonintyping, phagetyping.

2. Serology (detection of antibodies to exotoxin A): CFT, PHAT.

Prevention and treatment. For specific prevention, an associated vaccine has been developed, including Pseudomonas antigens, Proteus, Staphylococcus. With localized burns, pyoimmunogen can be used.

In therapy, antibiotics are used, to which the isolated cultures are sensitive (more often – carbenicillin, gentamicin, amikacin, cefsulodin), as well as heterological immunoglobulin (locally), pyoimmunogen (for burns), immune plasma, immunoglobulin. A good effect for the treatment of purulent-septic processes is the use of bacteriophage, autovaccine.

Laboratory algorithms:

Algorithm: *"The course of microbiological analysis of a material for suspected Pseudomonas aeruginosa infection."*

The purpose of the study: isolation and identification of Pseudomonas aeruginosa from pathological material and objects of the environment.

Day 1: seeding on nutrition media: trypticase soy agar, trypticase soy broth, Mueller-Hinton agar, Drigalski, Hektoen, sugar broth, 5 % blood agar, eosin-methylthionine blue agar, Mac Conkey agar, Endo agar. Incubation at 42 ºC for 20–24 hours and at 37 ºC for 20–24 hours.

Day 2: accounting for results of growth on Endo medium (pale pink colonies – lactose negative). In the presence of a blue-green pigment, it is possible to finally identify P. auruginosa, for non-pigmented colonies, carry out transfer to King A medium and put additional tests: seeding on Hugh-Leyfson medium with incubation in aerobic and anaerobic conditions, on the medium with gelatin, and also for detection the ability to grow at 42° and 5 °C on simple nutrient medium (for differentiation P. aeruginosa and P. fluorenscens).

If there is no growth or doubtful results on solid media, the tubes with broth with the growth of culture are picked up and transfer on agar in Petri dishes.

Day 3: accounting for results of growth. From the slant agar, a smear is prepared, stained after Gram (Gram-negative rods). Take into account the splitting glucose with the formation of acid in aerobic conditions, the dilution of gelatin. Determination of P. aeruginosa growth at 42 ºC and absence of growth at 5 ºC. Conduct a test on cytochrome oxidase (positive test).

The serological method is aimed at the detection of specific antibodies to the antigen of P. aeruginosa using CFT, PHAT.

Algorithm: *"Determination of oxidation and fermentation of glucose on the Hugh-Leyfson medium"*.

The medium is poured into test tubes of 5–6 ml, sterilized at 120 °C for 10 minutes. The culture under study is seeded in two tubes by the injection (not reaching the bottom of the tube), vaseline oil is put into one sterile tube in a layer of 1 cm. After 24 hours of incubation at 37 °C, the result is taken into account: the absence of a change in the initial green color of the medium in both test tubes indicates the absence of oxidation and fermentation glucose; the appearance of yellow coloration of the medium only in the test tube without vaseline indicates the oxidation of glucose; the appearance of yellow coloration of the medium under vaseline oil indicates the fermentation of glucose.

Practical tasks, being carried out during practical classes:

1. Studying morphology of Klebsiela, Proteus and P. aeruginosa (in atlas and microslides).
   - Staining and microscopy of smears (by Gram and Burry-Gins).
   - Primary seeding of pathogenic material on Endo, Ressel, Hiss media.
   - Determination of cultural properties of Klebsiella, Proteus, P. aeruginosa.
   - Determination of the enzymatic properties of Klebsiella, Proteus, P. aeruginosa on Ressel, Hiss, and Endo media.
   - Determination of cytochrome oxidase after Kovach.
   - Determination of oxidation and fermentation of glucose on the Hugh-Leyfson medium.
   - Slide agglutination reaction with K-sera for differentiation of K. ozaenae and K. rhinoscleromatis and O-agglutination.
2. Studying biological preparations for serological methods (antigens and diagnostic sera).
3. Studying the scheme of laboratory diagnosis of Klebsiella, Proteus, P. aeruginosa infections.

Therminology: Enterobacteriaceae, Klebsiela pneumoniae, K. oxytoca, K. rhinoscleromatis, K. ozena, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa.

Theoretical questions for control:

1. Genus Klebsiella, major characteristics, antigenic structure.
2. Genus Proteus, major characteristics, antigenic structure.

3. Genus Pseudomanas, major characteristics, antigenic structure
4. Morphology and biological properties of opportunistic bacteria: Klebsiella, Proteus, Pseudomonas aeruginosa. International Classification. Resistance to environmental factors.
5. Culture properties of Klebsiella, Proteus, P. aeruginosa.
6. Routes of transmission and pathogenesis of Klebsiella, Proteus, P. aeruginosa infections.
7. The role in human pathology. Pathogenesis and clinical manifestations. Immunity.
8. Laboratory diagnosis of Klebsiella, Proteus, P. aeruginosa infections.
9. Treatment and control of Klebsiella, Proteus, P. aeruginosa infections.

Test tasks for control:

1. Swab from the oral mucosa of the patient with chronic stomatitis was sent to the bacteriological laboratory. If you suppose that the pathogen can be K. pneumoniae, which of the following methods of staining should be used to identify the capsule?

A. Burry-Gins. *D. Ozheshko.*
B. Ziel-Neelsen. *E. Gansen.*
C. Gram.

2. The bacteriological laboratory received a material for research from a patient with maxillofacial trauma with purulent-inflammatory complications caused by K. pneumoniae. Which of the following methods should be used for laboratory diagnosis of the disease?

A. Biological, microscopy. *D. Bacteriological, biological.*
B. Bacteriological, microscopy. *E. Serological, allergic.*
C. Allergic, biological.

3. The pus from the patient with otitis is delivered to a bacteriological laboratory. It is known that the primary seeding is carried out by the method of Shukevich. What medium should be chosen?

A. Endo. *D. MPA.*
B. Blood agar. *E. MPB.*
C. Sugar broth.

4. In the examination of pus on nutrition agar, round S-colonies grew, with a bluish-gray color, a pearlescent shade and a smell of jasmine. For what kind of microorganism is characteristic such a cultural property?

A. Pseudomonas aeruginosa. *D. Klebsiella pneumoniae.*
B. Citrobacter freundii. *E. Proteus mirabilis.*
C. Yersinia enterocolitica.

5. It is known that for the identification of the opportunistic microorganisms of the family of intestinal bacteria, serological reactions are used. What are these serological reactions?

A. NT. *C. HAIT.* *E. AT.*
B. PHAT. *D. PT.*

6. During examination of the sputum of a patient suffering from pneumonia a lot of Gram-negative rod-shaped capsule-producing bacteria were detected. What microorganisms are revealed in the sputum?

A. Escherichia coli. *D. Staphylococcus aureus.*
B. Neisseria gonorrhoeae. *E. Klebsiella pneumoniae.*
C. Neisseria meningitidis.

7. Green pigment of P. aeruginosa is demonstrated on:

A. L-J medium. *D. Serum agar.*
B. Bordet-Gangou medium. *E. Wilson Blair medium.*
C. Hiss medium.

8. "Swarming growth" is seen with:

A. Streptococcus pyogenes. *D. Proteus mirabilis.*
B. Bordetella pertusis. *E. Pseudomonas aeruginosa.*
C. Klebsiella pneumoniae.

9. Capsule is best demonstrated by:

A. Simple method. *D. Negative stain with Indian ink.*
B. Silver impregnation method. *E. Differential method*
C. Romanovsky-Gimsa's method.

10. Which of the following is not true about Klebsiella?

A. Gram-negative rods. *D. Sporeforming.*
B. Nonmotile. *E. Capsulated.*
C. Facultative anaerobe.

11. Bacterial capsule of K.pneumoniae is chemically composed of:

A. Polypeptides. *D. Polynucleotides.*
B. Hyaluronic acid. *E. Polysaccharides.*
C. Fatty acids.

Answers to test: 1 – A. **2** – B. **3** – D. **4** – A. **5** – E. **6** – E. **7** – D. **8** – D. **9** – D. **10** – D. **11** – E.

Yersinia enterocolitica

Taxonomy.

Family: Enterobacteriaceae

Genus: Yersinia

Species: Yersinia pestis, Yersinia pseudotuberculosis, Yersinia enterocolitica

The genus Yersinia belongs to the family of Enterobacteria and includes 11 species, three of which are pathogenic to humans:

- Yersinia pestis – causative agent of plague;
- Yersinia pseudotuberculosis – causative agent of pseudotuberculosis;
- Yersinia enterocolitica – causative agent of intestinal yersiniosis.

The latter two species are refered to opportunistic microbes, are sapronoses and cause diseases yersiniosis. In Ukraine, they account for 6–11 % of all intestinal infections. Y. enterocolitica and Y. pseudotuberculosis are widespread in nature, they are able to multiply in water, soil, plants. Usually they live in rodents, often found in pets.

Morphological and tinctorial properties. Y.enterocolitica is small motile Gram-negative rod with rounded ends. The average size is 0.8–1.2 × 0.3–0.7 microns, but in older cultures they may be longer and have a kind of thread. Motility is determined in cultures grown at 18–20 °C. They do not form spores and capsules.

Cultural properties. Facultative anaerobes. Y. enterocolitica grows well on simple nutrient media. The most favorable temperature for growth is 22–28 °C, pH 7,2–7,4. They grow on Endo agar, forming small, tender, convex, shiny, transparent, non-colored or slightly pink colonies with blue hue.

On MPA, they form small, shiny, colorless colonies with an entire margin (dewdrops), which increase with the extension the growing periods (at 22–25 °C). When cultivated at 37 °C, the colonies are opaque, have an uneven scalloped edge and a convex center. On MPB – uniform turbidity, then a precipitate and a film appear on the surface of the medium. They may grow at high content of sodium chloride in the medium (up to 4 %).

Enzymatic properties. Y. enterocolitica has a pronounced biochemical activity. They ferment glucose, maltose, mannitol without formation of gas, do not ferment sucrose, lactose, do not form hydrogen sulfide. The formation of indole is not constant. There are 5 chemovars. The basic biochemical features required for identification: splitting of urea, fermentation of sucrose, absence of fermentation of rhamnose, production of ornithine decarboxylase.

Antigenic properties. Y.enterocolitica has O- and H-antigens. More than 30 serovars are identified on the O-antigen. Pathogens of human diseases most often belong to serovars 09, 03, 05, 08. There are antigens of virulence – V and W. Common antigens are found with Salmonella, Shigella, V. cholera, Proteus, Hafnia.

Resistance. Y.enterocolitica is unstable to high temperature. At 100 °C, they die instantly, when heated to 60–80 °C – after 15–20 min. They are well

preserved at low temperature (-15–20 °C) and actively propagating in food products that are stored in refrigerators and vegetable stores; at 4–14 °C bacteria are not only stored, but multiply. Direct sunlight kills them for 30 minutes, scattered – in 6–8 hours. They die quickly when dry. In food products Y.enterocolitica can persist and even multiply. In the water of open reservoirs and soil they can exist for 1 month or more.

Y. enterocolitica live in food: in milk – up to 18 days, in bread, confectionery – up to 16–18 days, in butter – up to 145 days. When ingested in ready-made dishes, especially from raw vegetables, they quickly multiply, creating a large concentration of bacteria. Bacteria release toxic substances, especially at low temperatures, which leads to the accumulation of not only bacteria, but also products of their metabolism – toxins.

Solutions of disinfectants (sulme, chloramine, phenol) kill bacteria in a few minutes.

Pathogenicity factors. Factors of adhesion, invasion, antigens of virulence (more actively synthesized at a temperature of 8–22 °C), enterotoxin. Enzymes of aggression: hyaluronidase, neuraminidase, urease, sIgA-protease.

Epidemiology. Laboratory animals are practically not sensitive to Y.enterocolitica. Under natural conditions, they can cause serious diseases of rodents, pigs, cats, dogs, often with fatal outcome.

The source of infection is more often sick animals (cattle, pigs, dogs, cats, birds), less often a human. The reservoir of the pathogen in nature is soil, water, plants.

Mechanism of transmission: fecal-oral, household contact, air-dust in the care of animals, meat and hides processing. Ways of transmission: food (meat, meat products, milk, vegetables, herbs, fruits) and water. They may be transmitted from person to person, may even be the cause of hospital infection.

Pathogenesis and clinical manifastation. Getting through the mouth in the gastrointestinal tract, Yersinia multiply in enterocytes and Peyer patches and migrate into the regional lymph nodes. Endotoxin and toxic substances produced by Yersinia cause the phenomenon of acute gastroenterocolitis. Concomitant regional lymphadenopathy simulates an appendicitis attack. When penetration of pathogens into the blood bacteremia and generalized process develop, in which various organs are affected: the liver, spleen, kidneys, lungs, joints.

The beginning of the disease is acute: general weakness, chills, headache, muscle aches, joints and abdominal pain, fever, nausea, vomiting, diarrhea, hyperemia of the skin of the face and neck (red hood syndrome), hyperemia and swelling of the skin of the hands (red mitral syndrome), hyperemia and swelling of the mucous membrane of the nasopharynx, tonsils. There are icteric, meningeal, and generalized forms.

Immunity. Antibodies of IgA class are produced on the first days of the disease and after healing the patient they circulate in his blood for several months, and in the chronic course of intestinal iersiniosis - much longer. A significant increase in IgA levels is characteristic for patients with reactive

arthritis, nodular erythema and other rheumatic diseases associated with the HLA-B27 genome.

Microbiological diagnostics. Material for the study: feces, vomit and washing of stomach, blood, urine, mucus from pharynx and nose, wound exudate, sectional material, food and water. Methods of research: bacteriological, microscopic, serological.

Prevention. Sanitary control of food storage and processing, rodent control. Compliance with the sanitary-and-hygienic regime at public catering establishments and personal hygiene rules. Specific prevention is absent.

Treatment. Antibiotics: levomycetin, cephalosporins III and IV generations (cefatoxin, ceftazidine, ceftriaxone), fluoroquinolones (abactal, norfloxacin, ofloxacin), aminoglycosides (gentamicin, sizomycin), chemotherapeutic drugs (bactrin).

Yersinia pseudotuberculosis

Causative agents of pseudotuberculosis are similar to those of the plague by morphological, culture and enzymatic properties. However, there are some differences. Bacteria of pseudotuberculosis are mobile, peritrichous. Biochemically more active.

Morphological and tinctorial properties. Y.pseudotuberculosis is rod, motile at a temperature below 37 ºC, has a bipolar staining, which is better detected in microslides from broth culture or in preparations from organs of dead animals. It forms a capsule. The size is 0.8–2 × 0.4–0.6 microns.

Cultural properties. It grows well on simple nutrient media. The optimum reproduction is 22–28 °C. At temperatures below 37 °C, on solid media it forms S-colonies, turbid with a grayish-yellow hue, at 37 °C – R-shaped, convex colonies with scalloped edges). On Endo medium the colonies are colorless in 48 hours at 37 °C. On liquid media uniform turbidity (S-form) or fluctuating sediment (R-form) are observed.

Enzymatic properties. They are biochemically active. The basic biochemical features required for identification are: urease production, rhamnose fermentation, lack of fermentation of sucrose, lack of indole production, negative reaction of Foges-Proskauer.

Antigenic properties. Y.pseudotuberculosis has flagellar H-antigen and two somatic antigens: smooth – typical and rough – group, common with antigen of the plague pathogen. By the ratio of O- and H-antigen, there are 13 serovars and subserovars. There are antigens of virulence - V and W.

Resistance. The pathogen is stable in the external environment: in water at room temperature it survives up to 1.5 months, at +4 ºC – up to 6 months, in vegetables and fruits it survives for several months. It has low resistance to heating at 60 °C, UV, to disinfectants.

Pathogenicity factors. Y.pseudotuberculosis is a highly invasive microbe. Adhesin (Yad A) promotes the attachment of bacteria to epithelial cells. Invasin is synthesized at 28–30 °C, thus participating in penetration of bacteria in the intestinal epithelium in the early stages of infection. Effector proteins (Yop) inhibit phagocytic activity and exhibit cytotoxic activity.

The thermolabile toxin has the effect of enterotoxin. Superanthigen (YPM) disrupts the function of epithelial cells, reducing the transport of ions and increasing the permeability of the epithelium, and also stimulates the proliferation of T lymphocytes and their activation, which leads to the synthesis of cytokines and, as a result, to the development of shock and tissue damage. SIgA proteases split secretory immunoglobulins of class A and suppress local mucosal immunity.

Epidemiology. To the causative agent of pseudotuberculosis animals are sensitive mainly: rodents, domestic and carnivores. The main carriers and the source of pseudotuberculosis are mouse-like rodents. The reservoir of the pathogen in nature: many types of mammals (cattle, cats), birds, rodents (mice, rats) which shed the microbes with feces, as well as water, soil, in which the accumulation of the microbe occurs. A human is infected by eating foods and water contaminated by the secretion of mouse-like rodents. Infection of a person from a patient or carrier does not occur. The natural susceptibility of human to the causative agent is high. The disease is widespread, occurs in the form of sporadic and epidemic outbreaks, and has seasonality (February-March).

Pathogenesis and clinical manifastation. Pseudotuberculosis is characterized by the polymorphism of the clinical picture and the cyclicity of the current. Y. pseudotuberculosis causes pericarditis, appendicitis-like syndrome, enterocolitis. Incubation period lasts from several hours to three weeks. In the clinical picture, the syndrome of general intoxication is in the foreground: weakness, headache, loss of appetite, muscle pain, joints, in severe cases – nausea, vomiting, loss of consciousness, excitation or inhibition.

Together with intoxication from the first days, there are symptoms of lesions of certain organs and systems, most often GIT. In case of a break of the lymphatic barrier bacteremia occurs, as a result of which the bacteria spread throughout the body, causing the formation of granulomas and microabscesses in the liver, spleen, lungs, joints. At the same time there is an allergy to the microorganism.

Immunity is unstable, nonsterile. Antibodies do not have protective activity. The body develops hypersensitivity.

Microbiological diagnostics. Material for research: feces, blood, bile, joint fluid, bronchial fluid, food, water. From the first days of the disease, mucus from the pharynx, urine on the first week, and faeces are studied throughout the illness. Bacteriological and serological methods are used. The material is placed in a phosphate buffer and enriched at 4 °C for 21 days, periodically seeded on a solid media (Endo, Serov).

Serologic examination is carried out at the 2nd week and 3–5 weeks by AT, PHAT and ELISA. Diagnostic titre is serum dilution 1 : 200.

For the diagnosis of pseudotuberculosis, an allergic method is used (pseudotuberculin – i/c, result after 48 hours, "+" for edema and hyperemia over 20 mm). Allergic sensitization develops at the beginning of the disease and persists for a long time.

Prevention. To prevent pseudotuberculosis, you must adhere to sanitary and hygiene rules, rules for cooking and storing food, especially in catering establishments. Do not use inadequately cooked or roasted meat, especially pork. Patients with diarrhea who are in hospital treatment, should be isolated before establishing the etiology of the disease.

Treatment. Y.pseudotuberculosis is susceptible to aminoglycosides, third generation of cephalosporins, fluoroquinolones, levomycetin, tetracyclines and trimethoprim / sulfamethoxazole. Fluoroquinolones are the drugs of choice for generalized forms of yersiniosis.

Serratia

Taxonomy.

Family: Enterobacteriaceae

Genus: Serratia

Species: S. marcescens, S. liquefaciens, S. rubidaea, S. plymuthica, S. proteamaculans, S. odorifera, S. ficaria, S. fonticola.

The bacteria were first isolated by the Italian bacteriologist B.Bizio and named them Serratia marcescens in honor of the pilot Serafino Serrati that conducted the vessels along the river Arno.

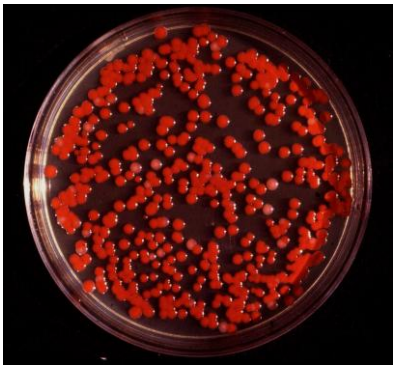
The genus Serratia is one of the oldest representatives of the Enterobacteriacea family. There are 10 species of Serratia, but only Serratia marcescens, S. rubidaea, S.liquefaciens are isolated in routine bacteriological laboratories. Previously, they were considered saprophytes, and only recently they have been isolated in hospital bacteremia, pneumonia, enteritis, urinary tract infections, suppuration of surgical wounds and skin lesions.

Morphological and tinctorial properties. Bacteria of the genus Serratia are straight, usually motile rods, a size of 0.9–2.0 × 0.5–0.8 microns, some strains have a capsule.

Cultural properties. Facultative anaerobes, almost all strains can grow at temperatures from 10 to 36 °C. They produce catalase. Different strains produce two different pigments: prodigiosine and pyrimin. Prodigiozine does not diffuse in the medium, insoluble in water, is produced by S. marcescens and by the majority of strains S. plymuthica, S. rubidaea, pigmenting colonies in red of different intensity (*Fig. 4A*). Color depends on the conditions of cultivation. To detect pigment bacteria should be cultivated on glycerol-peptone agar at 20–35 °C.

Pyrimin is soluble in water, diffuses in the medium. On blood agar at 37 °C, S. marcescens forms a grayish-white transparent S-colony 1–2 mm in diameter, which can be smooth or fine-grained (*Fig. 4B*). At room temperature, after 24–48 hours, the colonies become red. On a slant agar bacteria form a smooth white deposit. Since most isolates do not ferment lactose, they form a colorless colony on Ploskirev medium.

Biochemical properties. All species break down glucose, mannitol, synthesize indole, recover nitrates.


A

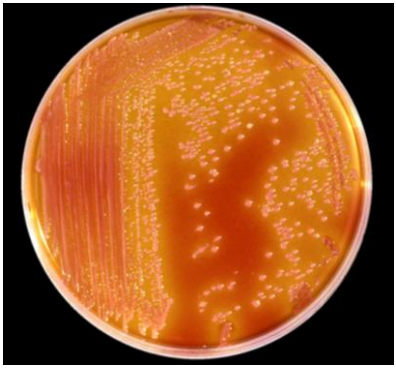

B

Fig. 4. Colonies of S. marcescens: A – on glycerol-peptone agar; B – on blood agar

Antigenic properties are only studied in S. marcescens. At present, the antigenic-diagnostic scheme of differentiation of these bacteria includes 21 O-antigen and 25 flagellar H-antigens. Some of them can give cross-reactions between themselves (O2 and O3, O6 and O7).

Resistance. Phenol, 1 % sodium hydrochloride, 70 % ethanol, formaldehyde, glutaraldehyde and chlorhexidine are effective against Serratia. Bacteria of the genus Serratia are inactivated by ultraviolet, gamma radiation, heated at 121 °C for 20 minutes and dry heat (165–170 °C for 2 hours).

Pathogenicity factors. Fimbria, hemolysins, sideroform system (which causes absorption of iron ions from blood and tissues), proteases (cause hemorrhages on the skin and mucous membranes) and thermolabile cytotoxin (its effect is similar to the action of the Schiga-like E. coli toxin).

Epidemiology. Serratia is found in the intestine of healthy people, animals, in soil, water, air, on plants, they are isolated from the defecation of insects and rodents. There are reports that S. marcescens can cause up to 10 % of hospital bacteraemia and pneumonia, 5 % of urinary tract infections, surgical wounds, and pustular skin lesions. Arthritis, endocarditis, osteomyelitis often occur in addicts.

Serratia are often passed through the hands of the medical staff. Most often they penetrate into the body through permanent catheters, intubation devices, as well as solutions for injections.

Pathogenesis and clinical manifastation. Coliform bacteria make up a large part of the normal intestinal aerobic flora, where they usually do not cause the disease, and on the contrary, they can participate in ensuring its normal functioning. These microorganisms become pathogenic only when they penetrate the tissues, especially in the urinary and biliary tract, lungs, peritoneum, or meninges, where they cause inflammation. In patients with immunodeficiencies, coliform bacteria can penetrate the bloodstream and cause sepsis. Serratia spp. is a common cause of secondary infections in hospitalized patients. Serratia spp. can cause pneumonia, bacteremia and endocarditis.

Laboratory diagnostics. The patient material for examination is taken depending on the localization of pathological processes. Most often it is blood, pus, urine, feces, bile, sputum. In the smears Serratia spp. have the form of Gram-negative rods, some strains have a capsule. Microbiological diagnostics is based on the isolation of pure cultures and the determination of their species by cultural properties and biochemical tests. Seedig is carried out on blood agar, the differential Endo medium, and especially MPA with DNase, toluidine blue and cephalothin. On the blood agar, S. marcescens and S. rubidaea form transparent grayish-white colonies, smooth or fine-grained. After 24–48 hours at room temperature they will produce a red pigment. On differential media colonies are colorless and slightly pink, smooth, slightly convex (*Fig. 5*). On these media, Serratia forms colonies, similar to colonies of Escherichia, but without metallic shine, with a reddish rim.

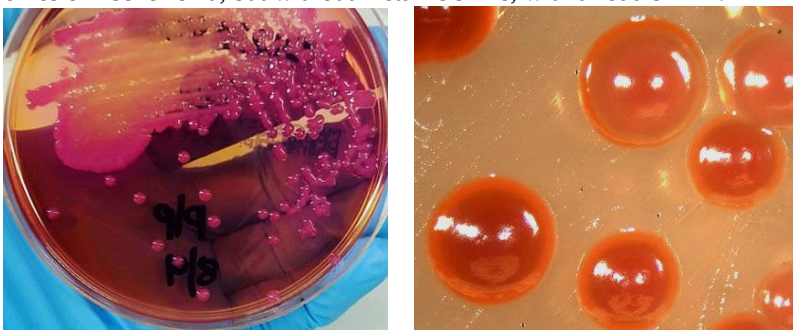
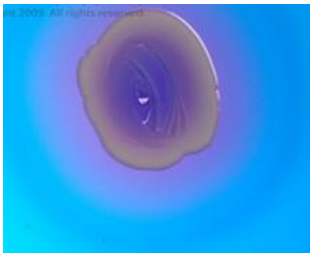


Fig. 5. Colonies of S. marcescens on Endo agar

On agar with DNase and toluidine blue Serratia form colonies with a characteristic blue border, while colonies of other enterobacteria do not have it (*Fig. 6*).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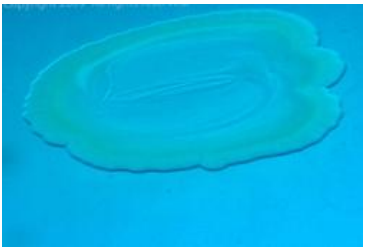
B

Fig. 6. Growth of S. marcescens (A) and E. coli (B) on agar with DNase and toluidine blue

Identification of isolated pure cultures is carried out solely by means of biochemical tests. Serratia constantly ferment glycerin, maltose, mannitol, salicin, sucrose and sorbitol. They split variably grow on citrate media such alcohols and carbohydrates as adonite, inositol, xylose and celibiosis.

To characterize Serratia the following features are important, such as the ability to decarboxylate lysine and ornithine, a positive reaction of Voges Proskauer, a negative reaction with methyl red, the ability to dilute gelatin, the presence of DNase, lipase. Identification is greatly facilitated, if isolated strains form pigment. Serologic typing of isolated strains of S. marcescens is carried out in slide agglutination reaction with O- and H-sera.

Prevention. The basis for prevention of acute intestinal infections is compliance with the rules of personal hygiene, food hygiene and water supply. In infant establishments it is important to strictly control the technology of cooking, transporting and realizing food.

Isolation of patients is carried out in the hospital in severe cases or at home. In the foci of infection, patients are monitored, and one carries out a bacteriological examination of persons who have had contact with the patients.

Treatment. In infections caused by hospital strains that produce beta-lactamases, all cephalosporins of II and III generations are ineffective. These strains tend to retain susceptibility to protected beta-lactams (piperacillin/tazobactam, cefoperazone/sulbactam), cephalosporins of IV generation, carbapenems. Fluoroquinolones are an alternative to beta-lactams. Aminoglycosides are generally used in combination with beta-lactams or fluoroquinolones.

Citrobacter

Taxonomy.

Family: Enterobacteriaceae

Genus: Citrobacter

Species: C. freundii, C. diversus, C. koseri, C. amalonaticus.

The genus Citrobacter includes 11 types of bacteria that are found in water, soil, food and other environmental objects. They constantly live in the intestine

of healthy people as representatives of normocenosis. Most species of Citrobacter are not pathogenic to human. The name of the genus Citrobacter is related to the ability of bacteria to dispose of citrate and use it as the only source of carbon.

Morphological and tinctorial properties. Gram-negative rods, usually motile due to peritrichially located flagella. They do not produce spores and capsules.

Cultural properties. Citrobacter spp. are the facultative anaerobes, grow well on simple nutrient media, forming convex colonies of 2–4 mm in diameter, which slightly opalesce. On liquid media they give homogeneous turbidity. Temperature limits of growth – 12–43 °C, optimum pH – 7,2. When growing on bismuth sulphite agar Citrobacter form greenish brown or black colonies and release volatile compounds with an unpleasant odor (*Fig. 7*).

Fig. 7. Colonies of Citrobacter on bismuth sulphite agar

Enzymatic properties. Bacteria of the genus Citrobacter ferment fructose, glucose, lactose, sorbitol, rhamnose, utilize citrate, give a positive reaction of Voges Proskauer. Oxidase-negative and catalase-positive.

Antigenic properties. Bacteria of the genus Citrobacter, as well as other enterobacteria, have O-, H-antigens, some species of the genus contain Vi-antigen, serologically identical to the Vi-antigen of S. typhi.

Resistance. Phenol, 1 % sodium hydrochloride, 70 % ethanol, formaldehyde, glutaraldehyde and chlorhexidine are effective against Citrobacter. Bacteria of the genus Citrobacter are inactivated by ultraviolet, gamma radiation, heated at 121 °C for 20 minutes and dry heat (165–170 °C for 2 hours).

Pathogenicity factors. Microcapsules, flagella, integrin (as invasive factors), surface protein adhesin (absent in avirulent strains), hemagglutinins, enzymes, thermostable and thermolabile enterotoxins.

Epidemiology. Citrobacteria are found in the intestine of healthy people, many animals, they can be found in soil, water, sewage, products. Some serotypes of Citrobacter can cause both sporadic diseases and outbreaks occurring in the type of acute gastroenteritis, dyspepsia, food toxicoinfections. The source of infection is people and animals. The mechanism of transmission is fecal-oral and contact.

The main route of transmission is nutritional, through milk, dairy products, butter, confectionery, meat of birds and animals. In weakened children of early age a household contact is possible through toys, objects of care, hands of the personnel. Bacteria can penetrate through burns, ulcers on the skin, intravenous catheters, surgical and diagnostic procedures in the gall bladder, gastrointestinal tract, genito-urinary organs. Lungs can be infected with intra-tracheal intubation or infected dust.

Nevertheless, hospital infections of gallbladder and urinary tract, otitis and osteomyelitis, especially in weakened people and newborns, are due to the horizontal transmission through the hands of the medical staff.

Pathogenesis and clinical manifastation. Bacteria colonize the oral cavity, intestine, or respiratory system. Then the infection can spread, causing bacteremia, sepsis, central nervous system and urinary tract infections, endocarditis, respiratory tract infections, abdominal abscesses, pneumonia. C.diversus and C.ferundii are frequent pathogens of meningitis and abscesses of the central nervous system. From patients with enteritis, urethritis, cholecystitis, osteomyelitis, otitis, endocarditis C.freundii and S.amalonaticus most often are isolated. Citrobacter spp. and Serratia spp. are a common cause of secondary infections in hospitalized patients. The mortality from infections caused by Citrobacter can reach 33–48 %.

Cytobacteriosis can occur in the form of food poisoning, gastritis, enteritis or gastroenteritis. Incubation period of citrobacteriosis is from 2 to 5 hours. Gastritis begins acutely with nausea, pain in the epigastric region. The body temperature, most often, ranges from 37–37.5 to 38–38.5 °C. Symptoms of poisoning are moderate. Duration – 1–6 days.

Laboratory diagnostics is based on isolation, biochemical and serological identification of the pathogen. The isolation of Citrobacteria from feces is complicated by the fact that the ability of bacteria to ferment lactose can mask them on McConkie and Ploskirev media. A more suitable is medium with 0.5% tyrosine, as only Citrobacteria can cause its enlightenment.

On Endo agar, strains that break down the lactose form pink colonies, but without metallic shine. On Ploskirev medium, lactose-positive colonies have a rich red color with a dark center, on bismuth-sulfite agar – brown or black colonies. In case of suspicion of Citrobacter it is recommended to seed on agar with cefsulodynum and novobiocin, on which bacteria form colonies with a red center and a transparent colorless periphery ("bullish eye") (*Fig. 8*).

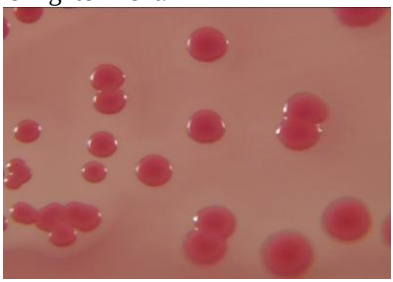

Fig. 8. Colonies of Citrobacter on agar with cefsulodynum and novobiocin

When seeding in Hiss medium, citrobacteria ferment glucose, lactose, mannitol, maltose, rhamnose, sorbitol, arabinose and xylose. They are able to utilize of citrate. They produce catalase, do not produce oxidase, give a negative reaction to Foges Proskauer, grow on Simons agar. On smears they have the appearance of small straight Gram-negative rods, arranged singly or in pairs.

Serologic identification is carried out in AT with polyvalent O-antiserum and with CiOA, CiOD and CiOF-sera, since the bacteria of these groups are most prevalent. The final identification is carried out with H-monoreceptor sera.

Prevention. Since Citrobacter spp. most commonly cause hospital infections, the source of which can be the gastrointestinal tract and the hands of medical personnel, non-specific prevention of infection is in compliance with the rules of personal hygiene, food hygiene and water supply.

Treatment. The approach to antimicrobial therapy is similar to that in the treatment of enterobacteria-induced diseases. Antibacterial agents active against citrobacter: ciprofloxacin, levofloxacin, aminoglycosides, chloramphenicol, imipinem and biseptol.

ENTEROBACTER

Taxonomy.

Family: Enterobacteriaceae

Genus: Enterobacter

Species: E. cloacae, E. sakazaki, E. agglomerans, E. aerogenes, E. gergovie, E. amnigenus, E. taylorae.

The name Enterobacter is proposed by E.Harmaeche and P.Edwards in 1960.

Morphological and tinctorial properties. Bacteria of the genus Enterobacter are Gram-negative rods, corresponding to the main position of the characteristics of the Enterobacteriaceae family, they are motile, in smears are single, less often short chains. Some strains form a capsule.

Cultural properties. Bacteria of the genus Enterobacter grow well on simple nutrient media that are used to grow enterobacteria. Facultative anaerobes. The temperature optimum is 30–37 °C, the optimum pH is 7.2. On solid media they form slimy and non-slimy colonies that resemble the colonies of Escherichia and Klebsiella. Lactose-positive strains form pink or raspberry colonies on media Endo, Ploskirev and McConkey. Lactose-negative strains form a yellowish colony. They cause turbidity of liquid media.

Enzymatic properties. Biochemical reactions of different species and bio-groups are significantly different. Bacteria of the genus Enterobacter do not form indole and hydrogen sulfide, do not possess deaminases of amino acids, utilize citrate, weakly hydrolyze urea, ferment a sorbitol, rhamnose, xylose, maltose, rafinoz, sucrose, are variable in relation to inositol, adonite, dulcit, salicin.

Antigenic properties. They have O- and H-antigen, the capsule strains also K-antigen; typing is performed on the O-antigen.

Resistance. All enterobacteria are characterized by high resistance to disinfectants.

Pathogenicity factors. The main factors are microvilli (facilitate colonization) and endotoxin. Strains of E.cloacae isolated from patients with hemolytic uremic syndrome produce Shiga-like toxin and superficial protein that inhibits the system of porins, which manifests in reducing the sensitivity to beta-lactam antibiotics. It was also established its identity with Yersinia enterocolitica invasive protein, serum resistance factor, and Salmonella typhymurium resistance factor to the internal microbicidal mechanisms of the macrophages.

Epidemiology. The value of Enterobacter in the pathology of humans and animals is not fully understood. E.agglomerans can cause opportunistic infections in weaked individuals, is often found after an invasive procedure. In the medical facilities, the pathogen can be transmitted through the hands of the staff. Quite often E. cloacae and E. aerogenes contaminate various solutions for intravenous administration.

Pathogenesis and clinical manifastation. The bacteria of the genus Enterobacter include 13 species. E.cloacae, E.aerogenes are more often isolated from sick people, E.gergovial, E.sakasaki much less often. Enterobacteria rarely cause independent infections. Most often, they infect patients with impaired immunity. Recently, enterobacteria are responsible for 15 % of all hospital infections, of which about 10 % are septicemia. Less often they cause meningitis, contaminate surgical burn wounds, affect the organs of the respiratory and genitourinary systems and the gastrointestinal tract.

The references describe the cases of the isolation of these bacteria from patients with acute gastrointestinal diseases, infections of the bile and urinary tract, with sepsis and hospital infections, purulent skin lesions. E. cloacae is most often isolated. Meningitis and bacteremia caused by E. sakazaki are described. The lesions caused by them do not have any pathognomic symptoms.

Microbiological diagnostics. Methods of diagnostics: bacteriological and microscopic. Most species can be identified by deoxycarboxylation of amino acids and fermentation of carbohydrates and antigenic structures.

Enterobacter spp. grow well on simple and differential diagnostic media such as Endo, Levin and Ploskirev. Lactose-positive strains form mucous or non-slime colonies. On Endo agar, they have a crimson or pink color with a metallic shine or without it. On Ploskirev medium, they have a yellowish tinge. Gram-negative rods are visible in the smears from the colonies; individual strains have a capsule. Identification of isolated pure culture is carried out according to biochemical properties.

The vast majority of strains ferment glucose, lactose, maltose, mannitol, sucrose, trehalose, do not emit indole and hydrogen sulfide, dilute gelatin, give positive Foges-Ploskauer reaction. Slide agglutination reactions with O- and H-sera have begun to be used.

Prevention. The basis for prevention of acute intestinal infections is compliance with the rules of personal hygiene, food hygiene and water supply. In infant establishments it is important to strictly control the technology of cooking, transporting and realizing food.

Treatment. Enterobacteria are characterized by multiple resistance (especially to ampicillin and cephalothin). The choice of drug depends on the results of the determination of sensitivity to antibacterial agents. The base of antimicrobial therapy are aminoglycosides, cephalosporins of the 3rd generation, fluoroquinolones.

Laboratory algorithms

Algorithm: *"Rules for sampling material for suspected infection, caused by opportunistic microorganisms of the Enterobacteria family".*

Material for examination: feces, vomit, pus, wound exudate, punctures, sputum, mucus from pharynx, nose, ear, urine, bile, liquor, blood, secretion of the genital organs, bits of tissue taken during surgery, sectional material, etc. Clinical material is taken before starting antibiotic therapy, adhering to the rules of asepsis.

Venous blood in a volume of 8–10 ml is introduced by piercing the cork (pre-treated with 70 % ethyl alcohol) into a hermetic bottle containing 80–100 ml of the nutrient medium for sterility control. The remaining blood in the syringe is used to prepare smears.

Pus is placed in a test tube with a medium "SCS-199" or, with its abundant amount, in a hermetic tube.

Wound and fistula exudate, after cleaning their surface from necrotic masses is selected with a cotton swab, which is immediately placed in the medium "SCS-199", or through a drainage tube with a sterile syringe.

Biopsy weighing not less than 0,5–1,0 g is taken from the depth of the wound using a sterile scalpel and immediately placed in a test tube with the medium "SCS-199".

Urine is collected in a sterile vial (20–30 ml). Liquor in a volume of 3–5 ml is placed in a sterile test tube, bile is taken at duodenal sounding and placed separately into the test tubes of A, B, and C. The sputum is collected in separate vials. Mucus from the parynx, nose, ears, secretion of the genital organs are selected with a cotton swab, which is placed in a test tube with a medium "SCS-199".

The material is delivered to the laboratory not later than 2 hours, keeping it at room temperature or at 37 °C. Compliance with the specified conditions of selection and delivery of material allows you to save and anaerobic microflora in a viable state.

Algorithm: *"The course of microbiological examination of a material for suspected infection, caused by opportunistic microorganisms of the Enterobacteriacea family".*

Microscopic examination is of presumptive importance, due to similarity of pathogens with other, in particular non-pathogenic, microorganisms. Microscopy of the native material is carried out for the purpose of the prompt receipt of information on the intensity of microbial contamination of the samples and the ratio of different microflora. Smears are stained after Gram. At microscopy, attention is drawn to the ratio of Gram-negative and Gram-positive bacteria, their shape, spores, capsules. Enterobacteria are Gram-negative rods, in some species with a capsule. The results of microscopy are compared with the data of the isolation of cultures on nutrient media.

Bacteriological examination. Enterobacteria are unpretentious microorganisms and grow well on universal media.

Stage I: The primary seeding of the material is carried out on a complex of universal media intended to isolate not only enterobacteria but also anaerobic bacteria and aerobes (5% blood agar, medium for anaerobes). In the study focused on enterobacteria selective solid media Endo, Levin, Ploskirev, McConkey, bismuth-sulfite agar (to isolate Citrobacter) and others are used. Liquid pathological material (pus, wound exudate, suspension of 10 % of biopsy samples, urine, homogenized sputum, etc.) is seeded in solid nutrient media by a standard bacteriological loop (diameter 3 mm) for semiquantitative determination of the concentration of bacteria (in 1 ml or 1 g).

Petry dishes with culture on 5 % blood agar and selective media are incubated at 37 °C for 24–48 hours. The material remaining after the seeding in the transport medium "SCS-199" is used to accumulate pathogens at 37 °C. In the absence of signs of growth of blood culture, Petry dishes are incubated for up to 6 weeks.

Stage II: the isolation of pure cultures and their primary differentiation. Studying the cultural properties and morphology of bacteria in all varieties of colonies grown on media; quantification of the number of bacterial species in 1 ml (g) of the test material. On 5 % of the blood agar enterobacteria form colonies of intermediate size, round, moist, sometimes rough, mostly without hemolysis; some strains of Serratia marcescens have a red pigment, bacteria of the genus Proteus are characterized by creeping growth.

On Endo agar, lactose-positive enterobacteria form red colonies, lactose-negative - colorless, turbid or transparent. Colonies with yellow pigment are characteristic for Enterobacter sakazaki and Enterobacter agglomerans. After microscopy of smears stained after Gram, the material from suspicious colonies is collected for the accumulation of pure culture on a slant MPA or a combined medium (Ressel, Cligler, three-sugar agar, Olkennitsky). The cultures are incubated at 37 °C for 18–24 hours.

Stage III: after receiving the growth and primary differentiation of enterobacteria on the policarbohydrate medium, the final identification of the culture and determination of its sensitivity to antimicrobial drugs is carried out. Such properties of microbial cultures as the ability to utilize various carbohydrates and citrate, to form acetone or a mixture of acids upon the fermentation of glucose (Foges-Proskauer tests and methylene red), urease activity, hydrogen sulfide and indole formation, decarboxylation and deamination of amino acids, motility, and the antigenic structure are determined.

The following morpho-physiological signs indicate the belonging a microorganism to the family of enterobacteria: Gram-negative rods with rounded ends, without spores; facultative anaerobes, form characteristic

colonies in 1–2 days at 30–37 °C; oxidase-negative, have catalase and nitrate reductase activity; ferment and oxidize glucose.

The etiological significance of the isolated opportunistic enterobacteria is determined on the basis of the source of their selection and concentration in the material. They have etiological significance at any concentration of bacteria isolated from blood, liquor, pleural fluid, closed cavities. Etiologically significant are enterobacteria isolated from wounds at a concentration of 10^5 cells/ml, from urine at a concentration of 10^4 cells/ml, from sputum at a concentration of 10^6 cells/ml, from tracheobronchial washings at a concentration of 10^4 cells/ml.

Serodiagnosis is sometimes used as an additional method.

Algorithm: *"The course of the microbiological examination of the material for suspected Y.enterocolitica infection."*

The purpose of the examination: the isolation and identification of Yersinia from the pathological material.

Day 1: Seeding on Endo and Levin media and buffering enrichment medium (pH = 7.2). Incubation at 20–28 ºC for 24h. The material that was in the phosphate buffer is subject to cold enrichment.

Seeding from the enrichment medium is carried out on 2, 3, 5, 7, 10 day by a loop from the upper third of the medium layer, but not from the surface.

Day 2: small, brilliant colonies are transplanted on combined Ressel medium, Petry dishes are left at 20–28 °C, and carry out seeding from the enrichment medium on Endo or Levin media.

Day 3: accounting for results of growth. Round, shiny, with a pink shade and an entire edge colony are seeded on Ressel medium. From Ressel medium a microslide is prepared and stained after Gram. In the presence of Gram-negative rods that do not ferment lactose, ferment glucose and urea, which do not form hydrogen sulfide, motility is determined (at 18–20 ºC and 37 ºC) and colonie is seeded in Hiss medium (mannitol, maltose, sucrose, rhamnose, gelatin, citrate).

Day 4: record of growth results in Petry dishes on combined media. In the selection of Gram-negative rods that do not ferment lactose, rhamnose, which do not form hydrogen sulfide, ferment glucose, mannitol, sucrose, motile at 22 °C and nonmotile at 37 °C, the answer is given: the isolation of Y. enterocolitica.

Serovar is determined in slide AT with diagnostic sera.

Serologic examination is carried out in PHAT with diagnostic titre 1 : 160, as well as AT, ELISA.

Algorithm: *"The course of a microbiological examination of a material for suspected Citrobacter infection."*

Day 1: The studied material is seeded on Endo, Ploskirev, Wilson-Blair media.

Day 2: Selection of suspicious colonies for further examination. On Endo agar, Citrobacter spp. form pink or red colonies, similar to colonies of Escherichia. Lactose-negative variants form colorless and grayish colonies, with a pink tint and a

darker center. On Ploskirev medium colonies are pinkish-red, with a dark center or colored in the tone of the medium. In some strains, which intensively produce hydrogen sulfide, colonies have a black center. On bismuth-sulfite agar colonies are light green, brown or black. The color of the medium under colonies does not change.

Selected colonies (at least three with a homogeneous appearance of growth or 2–3 of each species) are seeded into test tubes with semisolid (0.2–0.3 %) agar, Klygler medium, Clark medium, and Simmons citrate agar. Under the cap of a test tube with semisolid agar, an indole indicator paper is placed. Cultures are placed in a thermostat at 37 °C for 18–24 hours.

Day 3: accounting for results of enzymatic activity. Cultures that ferment glucose and lactose or do not ferment lactose, utiliz citrate (growth on Simmons agar), form or do not form indole and hydrogen sulfide, give a positive reaction with methyl red and negative Foges-Proskauer are suspected for Citrobacter. To confirm the genus and the species, they are seeded on medium with amino acids: lysine and ornithine, the Hiss medium with adonine and the medium with sodium malanate, as well as slant MPA for further serological typing.

Day 4: the results of the examination are analyzed on the media seeded the day before. In the case of the isolation of cultures C.freundii conduct serological typing on the O- and H-antigens in the slide agglutination reaction, first with polyvalent, and then with typical and factor O-sera. After determining the O-antigen typing with H-sera is performed.

Algorithm: *"The course of a microbiological study of a material suspected for Enterobacter infection."*

On Day 1, the material is seeded on Petry dishes with Endo and Ploskirev media, and placed in a thermostat at 37 °C for 18–24 hours.

On the 2nd day the Petry dishes are looked over and colonies are taken for further examination. In these media Enterobacter form colonies of the usual size, mucous and non-slimy, resembling Escherichia or Klebsiella, with a metallic shine or without it, pink or raspberry. In case of growth of heterogeneous colonies at least 3 colonies are remouved, in case of growth of different colonies 2–3 colonies of each species are taken. Selected colonies are seeded on short Hiss medium, Klygler medium, Preuss medium with urea, Simmons citrate agar and Clark agar. Under the cap of test tube with semisolid agar, an indole indicator paper is placed. Cultures are incubated in a thermostat at 37°C.

On 3rd day, we analyze the results of growth on Hiss medium. Motile cultures that ferment or do not ferment lactose, do not form indole and hydrogen sulfide, do not hydrolyze or slightly hydrolyze urea that grew on Simmons agar, form acetylmethylcarbinol and give a negative reaction to methyl red, are suspected for Enterobacter. To determine the species isolated culture is seeded on the medium with amino acids: lysine, ornithine, arginine.

On the 4th day, the results of decarboxylation of amino acids are analyzed.

REFERENCES

1. Microbiology. Handbook to laboratory classes in microbiology / A. Ya. Tsyganenko, I. L. Dikiy, V. L. Tkachenko et al. – Kharkiv : Osnova, 2005. – 210 p.

2. Murray P. R. Manual of Clinical Microbiology / P. R. Murray, K. S. Rosenthal, M. A. Pfaller. – 8th Ed – Washington : ASM Press, 2015. – 848 p.

3. Ananthanarayan. Textbook of Microbiology / Ananthanarayan, Paniker. – 9th Ed – Orient Blackswan, 2013. – 657 p.

ADDITIONAL REFERENCES

1. Manual of Clinical Microbiology / C. Carroll Karen et al. – 10th Edition. – Vol. 2. – Washington, DC : ASM Press, 2011. – 2630 p.

2. Opportunistic Infections caused by Pseudomonas aeruginosa. Todar's Online Textbook of Bacteriology. – 2005 – Available at: http://textbookofbacteriology.net/pseudomonas.html.

3. Greenwood D. Medical Microbiology / D. Greenwood, R.C.B. Slake, M. Barer, L. Irving. – 18th ed. with studentconsult online access – Churchill Livingstone – 2012. – 794 p.

4. CDC. Klebsiella pneumoniae in Healthcare Settings. – Access: https://www.cdc.gov/HAI/organisms/klebsiella/klebsiella.html

Навчальне видання

ЗБУДНИКИ ОПОРТУНІСТИЧНИХ ІНФЕКЦІЙ

Методичні вказівки з дисципліни «Мікробіологія, вірусологія та імунологія» для студентів II і III курсів медичного та стоматологічного факультетів з англійською мовою викладання

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Комп'ютерна верстка О.Ю. Лавриненко

Формат А5. Ум. друк. арк. 2,3. Зам. № 18-33626.

Редакційно-видавничий відділ
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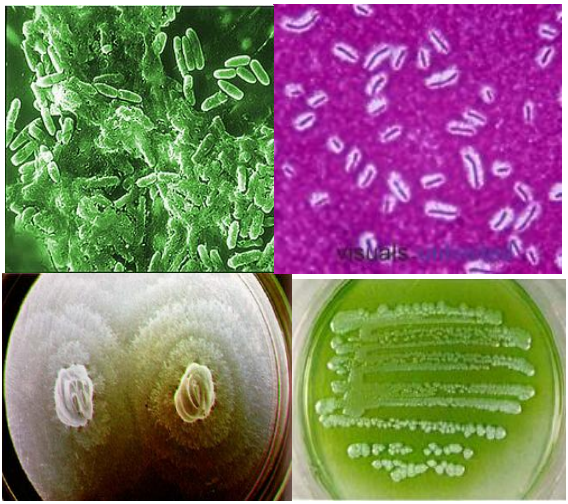
Свідоцтво про внесення суб'єкта видавничої справи до Державного реєстру видавництв, виготівників і розповсюджувачів видавничої продукції серії ДК № 3242 від 18.07.2008 р.



CAUSATIVE AGENTS OF OPPORTUNISTIC INFECTIONS

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology)

ЗБУДНИКИ ОПОРТУНІСТИЧНИХ ІНФЕКЦІЙ

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