



NORMAL MICROFLORA OF ORAL CAVITY AND MICROFLORA IN PATHOLOGICAL PROCESSES

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

НОРМАЛЬНА МІКРОФЛОРА ПОРОЖНИНИ РОТА ТА МІКРОФЛОРА ПРИ ПАТОЛОГІЧНИХ ПРОЦЕСАХ

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

**МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
Харківський національний медичний університет**

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Learning guide is related to the program of Ministry of Health of Ukraine and is recommended to students of the Faculty of Medicine and the Faculty of Dentistry of high medical schools of III-IV level accreditation.

Learning guide includes sections of medical microbiology that studies microflora of the oral cavity and its role in pathological processes. Characteristics of the main species of microorganisms of normal flora of the oral cavity, and pathogenic bacteria and viruses as well as the most modern information on methods of microbiological research are represented.

NORMAL MICROFLORA OF ORAL CAVITY AND MICROFLORA IN PATHOLOGICAL PROCESSES

Theme topicality.

Dental diseases are among the most common diseases of the human body. The microflora of the oral cavity is specific, unlike the microflora of other cavities in composition, quantity and function. Resident microflora plays an important role in maintaining the physiological state of the oral cavity, and in the development of dental diseases. In addition, lesions of the oral mucosa can be caused by various infectious agents of bacterial, viral and fungal etiology.

Goal:

– general: studying the main representatives of the resident microflora of the oral cavity, the peculiarities of the distribution of bacteria in different habitats of the oral cavity; consider the role of resident microflora in caries, periodontal diseases and purulent-inflammatory processes of the oral cavity. Studying the main pathogens of bacterial, viral and fungal stomatitis.

Concrete goals:

a) Students should know:

1. The main representatives of the resident microflora of the oral cavity, the role of normal microflora, features of the formation of the microbiocenosis of the oral cavity, age-related changes in the microflora.
2. Features of the composition of the microflora in caries and periodontal diseases, odontogenic infections, properties of cariogenic and periodontal microorganisms.
3. Characteristics of the main representatives of pathogens of bacterial, viral and fungal stomatitis.
4. Microbiological methods of research of microflora in caries, periodontal diseases, odontogenic infection and bacterial, viral and fungal stomatitis.

b) Students should be able to:

1. Follow the rules of work and safety with infected material, cultures of microorganisms, equipment.
2. Prepare and stain after Gram method smears from plaque, oral fluid, smears-imprints from the mucous membrane or elements of the lesion.
3. Examine the material using microscopic and bacteriological methods.
4. Evaluate the results of the study.

Equipment: museum micropreparations of residents of the oral cavity, microscope, immersion oil, disinfectant solution, cultures of microorganisms on nutrient media in Petri dishes, tables, atlas of microorganisms, presentation, guidelines.

Normal and resident microflora of the oral cavity. The role of normal oral microflora

Microflora of the oral cavity (syn. Microbiocenosis of the oral cavity) is a set of representatives of different taxonomic groups of microorganisms that inhabit the oral

cavity as a kind of ecological niche of the human body, entering into biochemical, immunological and other relationships with the macroorganism and with each other.

The permanent microflora of the human oral cavity is formed due to the mutual adaptation of the macroorganism and microbes. Interrelated adaptive changes lead to a biological "balance" between the human body and the microbial flora, and between its species. This "equilibrium" is dynamic. For example, it can be significantly disturbed during teeth whitening, when the number of microorganisms can vary significantly even during one day with the stability of the species composition of the microflora. However, after these short-term changes, the ecosystem of the oral cavity recovers very quickly, returning to a certain average equilibrium state and is disturbed significantly only as a result of effects that reduce the protective functions of the body. Violation of the general reactivity of the human body and the barrier functions of mucous membranes and skin can cause such changes in the composition and properties of the flora adapted to the macroorganism, which lead to autoinfectious processes and dysbacteriosis.

The microflora of the oral cavity is classified into autochthonous (resident, permanent) and allochthonous (transient, temporary).

Resident microflora consists of relatively constant species of bacteria characteristic of a particular habitat and age of a human, and it is able to recover quickly in case of its violation. The autochthonous microflora is divided into obligate, which constantly lives in the oral cavity, and facultative, in which opportunistic bacteria are more common. Opportunistic species are less common, they are most characteristic of certain diseases of the teeth, periodontium, oral mucosa and lips.

Transient microflora consists of non-pathogenic or opportunistic microorganisms that inhabit the oral cavity for a limited period of time without causing disease. However, in case of disturbances or death of the resident microflora, the representatives of the transient flora may occupy a niche of a particular habitat, which is vacated, that may further contribute to the development of pathology. The allochthonous microflora of the oral cavity is represented by microbes characteristic of other parts of the body; it consists of species that usually live in the intestines or nasopharynx.

Conditions for reproduction and long delay of microorganisms in the oral cavity are: temperature optimum, a large amount of moisture, neutral reaction of the environment, the anatomic features promoting accumulation of microbial cells.

The role of normal oral microflora

1. It stimulates the development of lymphoid tissue.
2. Due to the antagonistic effect it inhibits the reproduction of various pathogenic species of bacteria that enter the oral cavity. Normal flora microbes can inhibit the reproduction of other species and genera of bacteria due to higher biological potential (short lag phase, higher reproduction rate), competition for food source, by changing

the pH, production of alcohols, hydrogen peroxide, lactic and fatty acids, etc. Representatives of the normal microflora synthesize lysozyme, bacteriocins that have bactericidal activity against foreign microorganisms.

3. It supports physiological inflammation in the mucous membrane and increases readiness for immune responses.

4. It promotes the body's supply of aminoacids and vitamins, which are secreted by microorganisms in the process of metabolism.

5. Wasteproducts of microorganisms can stimulate the secretion of salivary and mucous glands.

6. Representatives of normal microflora are the causative agents and main factors of major dental diseases.

Characteristics of normal microflora

Bacteria occupy a dominant place both in terms of the diversity of species living in the oral cavity and in terms of their number (Table 1), although viruses, fungi and protozoa are also part of the oral microflora. 250-280 species of bacteria found in the oral cavity were isolated in pure culture and their properties were studied. It is believed that the normal ratio of anaerobic and aerobic microorganisms in the oral cavity is 10: 1. Bacteria with anaerobic type of respiration make up about 75 % of all bacterial flora.

Table 1. The main groups of bacterial microflora of the oral cavity

Respiration	Morphology	Genus
Obligate anaerobes	Gram-negative cocci	Veillonella
	Gram-positive cocci	Peptococcus Peptostreptococcus
	Gram-negative rod-shaped bacteria	Leptotrichia Bacteroides Fusobacterium Porphyromonas Prevotella
	Gram-positive rod-shaped bacteria	Bifidobacterium Propionibacterium
	Spirochetes	Treponema Borrelia
Aerobes and facultative anaerobes	Gram-negative cocci	Neisseria
	Gram-positive cocci	Staphylococcus Streptococcus
	Gram-positive rod-shaped bacteria	Corynebacterium Lactobacillus
	Filamentous bacteria	Actinomyces
	Spirochetes	Leptospira

1. Bacterial flora of the oral cavity

Cocci

Streptococci (genus *Streptococcus*).

Approximately 30–60 % of all oral microflora are facultative and obligate anaerobic streptococci, which belong to the family Streptococcaceae.

Streptococci are Gram-positive spherical bacteria that are arranged in chains or in pairs (*Fig. 1*). Immobile, do not produce spores; some form capsules. Special nutrient media (blood agar, sugar broth) are necessary for cultivation. In the environment, they are less resistant than staphylococci.

Streptococci are the main inhabitants of the oral cavity (1 ml of saliva contains up to 10^8 – 10^{11} streptococci). With significant enzymatic activity, streptococci ferment carbohydrates to form lactic acid. Acids that appear as a result of fermentation, inhibit the growth of a number of putrefactive bacteria that occur in the oral cavity. In addition, acids formed by streptococci lower the pH in the oral cavity and promote the development of caries. Also important is the ability of streptococci to synthesize insoluble polysaccharides from sucrose.

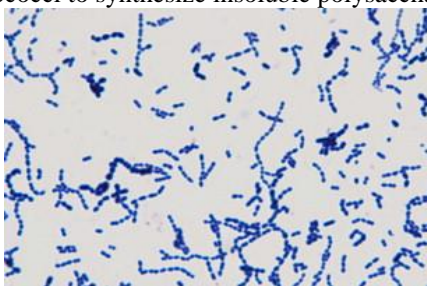


Fig. 1. Cells of *S. mitis*. Gram stain

Streptococci of the oral cavity are a special ecological group and are called "oral". These include the following species: *S. mutans*, *S. salivarius*, *S. sanguis*, *S. mitis*, *S. oralis* and others. Oral streptococci differ from each other in their ability to ferment carbohydrates and form hydrogen peroxide. They form colonies surrounded by a green zone of α -hemolysis on blood agar (*Fig. 2*). Colonization by oral streptococci of different parts of the oral cavity has qualitative and quantitative variations depending on living conditions. *S. salivarius* and *S. mitis* are present in the oral cavity in 100 % of cases. *S. mutans* and *S. sanguis* are found in large numbers on the teeth, and *S. salivarius* - mainly on the surface of the tongue. *S. mutans* and *S. sanguis* were detected in the oral cavity only after damage to the teeth.

Staphylococci (genus *Staphylococcus*).

Staphylococci are Gram-positive cocci. In pure culture they are located in the form of clusters resembling grapes, and in pathological material - in small clusters of cocci (*Fig. 3*). Non-motile. Facultative anaerobes.



Fig. 2. Colonies of *S. mitis* on blood agar

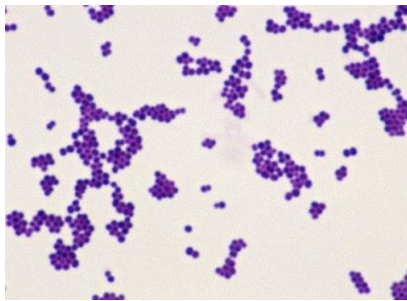


Fig. 3. Cells of staphylococci. Gram stain

They are part of the normal microflora of the human body, living in the nasopharynx, oropharynx and skin. Staphylococci in the mouth of a healthy person occur in an average of 30 % of cases. In dental plaque and on the gums of healthy people *S. epidermidis* is present mainly. In some people, *S. aureus* (the most pathogenic species) can be found in the oral cavity.

Possessing significant enzymatic activity, staphylococci are involved in the breakdown of food residues in the oral cavity. Pathogenic staphylococci (coagulase-positive), found on the nasopharyngeal mucosa and in the oral cavity, are common cause of endogenous infections, causing various purulent-inflammatory processes in the oral cavity.

Peptostreptococci (genus *Peptostreptococcus*)

Peptostreptococci are cocci arranged in pairs or chains. Non-motile. Obligate anaerobes. Poorly fermented carbohydrates. They grow on complex nutrient media with the addition of blood.

The following species are found in the oral cavity: *P. anaerobius*, *P. magnus*, *P. micros*. Peptostreptococci cause purulent-inflammatory diseases of different localization in association with other microbes.

Peptococci (genus Peptococcus)

Peptococci are cocci, arranged in pairs, tetrads, in the form of irregular clusters or short chains. Non-motile. Obligate anaerobes. They are demanding to nutrient media, grow better in the presence of fatty acids. Peptococci have weak polysaccharide activity, break down peptones and amino acids.

Peptococci are most often found in associations with fusobacteria and spirochetes in deep pulpitis, periodontitis, abscesses of the maxillofacial region. A typical species is *Peptococcus niger*.

Veillonella (genus Veillonella)

Veillonella are Gram-negative cocci that are arranged in pairs or, less frequently, singly, sometimes in small clusters (*Fig. 4*). Non-motile. Obligate anaerobes. They grow poorly on nutrient media, but their growth is markedly improved by the addition of lactate, which is a source of energy for them. They decompose low-molecular-weight carbohydrate metabolism products - lactate, pyruvate, acetate to CO₂ and H₂, helping to increase the pH of the environment.

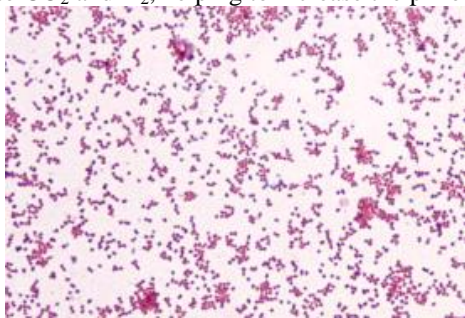


Fig. 4. Cells of *V. parvula*. Gram stain

The concentration of veillonella (species *V. parvula*) in saliva is approximately the same as green streptococci. In the mouth of healthy people, they are constantly present in large quantities (in 1 ml of saliva to 10⁷–10¹¹). They ferment acetic, pyruvic and lactic acids well to carbon dioxide and water and, thus, neutralize the acidic products of metabolism of other bacteria, which allows them to be considered as antagonists of cariogenic bacteria. Independently, usually they do not cause the development of pathological processes, but may be a part of mixed groups of pathogens. Their number increases with inflammatory processes, with odontogenic abscesses of the oral cavity.

Neisseria (genus Neisseria)

Neisseria are Gram-negative diplococci arranged in a pair of coffee beans facing each other with concave surfaces. Non-motile. Aerobes.

Neisseria are always found in large quantities in the mouth of healthy people (up to 1–3 million in 1 ml of saliva). Neisseria actively reduce oxygen, which decreases the redox potential of the environment and creates conditions

for the development of anaerobic microflora. There are pigment-forming species and species that do not produce pigments. The latter are most often found in the pulp and periodontium in acute serous inflammation and catarrhal inflammation of the oral mucosa.

Rod-shaped forms of bacteria

Lactobacilli (genus Lactobacillus).

Lactobacilli are Gram-positive rods of various lengths with rounded ends, often form short chains (*Fig. 5*). Sometimes mobile (peritrichia). They do not form spores and capsules. Facultative anaerobes, microaerophiles, less often - obligate anaerobes.

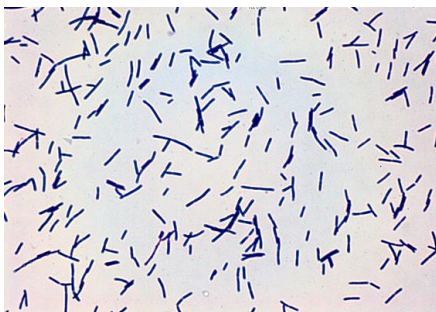


Fig. 5. Cells of lactobacilli. Gram stain

Lactobacillus acidophilus, *L. fermentum*, *L. brevis*, *L. casei* are the most common in the oral cavity.

Lactobacilli cause lactic acid fermentation. Due to the formation of large amounts of lactic acid, they delay the growth (are antagonists) of pathogenic, putrefactive and gas-forming microflora, but on the other hand contribute to the development of caries. The number of lactobacilli in the oral cavity during caries increases and depends on the size of carious lesions. Bacteria are able to exist at low pH values and, synthesizing a large number of acids, intensify the carious process. These microbes play a crucial role in the destruction of dentin after deformation of the enamel.

Bifidobacteria (genus Bifidobacterium).

Bifidobacteria are Gram-positive polymorphic rods, usually slightly curved or branched (often in the form of the Latin letters "Y", "X"), often with thickenings at the ends (*Fig. 6*). Non-motile, do not form spores. Obligate anaerobes. In addition to the oral cavity, bifidobacteria also inhabit the intestines. Bifidobacteria ferment various carbohydrates with the formation of organic acids, as well as produce B vitamins and antimicrobial substances that inhibit the growth of pathogenic and opportunistic microorganisms. In addition, they readily bind to epithelial cell receptors and form biofilms, thereby preventing colonization of the epithelium by pathogenic bacteria.

Propionibacteria (genus *Propionibacterium*).

Propionibacteria are polymorphic irregularly shaped rods, there are coccoid and slightly branched forms. Arranged singly, in short chains or small clusters. Gram-positive. Non-motile. Facultative anaerobes but grow better in anaerobic conditions.

Bifido- and propionibacteria are antagonists of pathogenic microflora; they are rarely isolated in purulent-inflammatory processes in association with other pathogens.

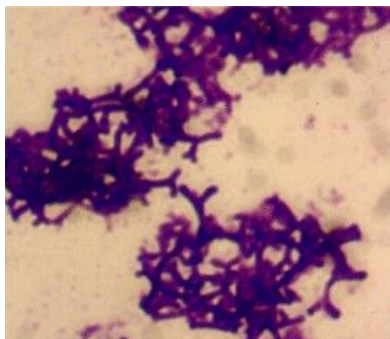


Fig. 6. Cells of propionibacteria. Gram stain

Corynebacteria (genus *Corynebacterium*).

Corynebacteria are straight or slightly curved rods, sometimes with club-shaped ends. Arranged: singly or in pairs, forming a V-shaped configuration or a stack of several cells located in parallel (*Fig. 7*). Gram-positive. They have grains of volute.

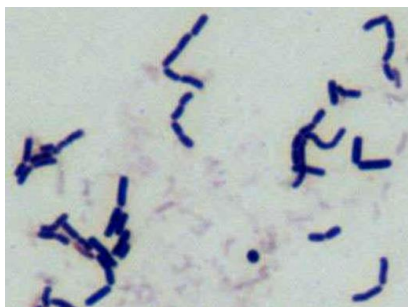


Fig. 7. Cells of corynebacteria. Gram stain

Corynebacteria are almost always found in large quantities in the mouth of a healthy person. These are non-pathogenic members of the genus. A characteristic feature of corynebacteria growing in the oral cavity is their ability to reduce the redox potential, which promotes the growth and reproduction of anaerobes.

Bacteria of the genera *Propionibacterium*, *Corynebacterium* and *Eubacterium* are often called "diphtheroids", although this is a more historical term. These three genera of bacteria belong to different families – *Propionibacteriaceae*, *Corynebacteriaceae* and *Eubacteriaceae*. All of them actively reduce molecular oxygen and synthesize vitamin K, which contributes to the development of obligate anaerobes. It is believed that some types of corynebacteria can cause purulent inflammation. Pathogenic properties are more pronounced in *Propionibacterium* and *Eubacterium* – they produce enzymes that affect the tissues of the macroorganism, often these bacteria are isolated in pulpitis, periodontitis and other diseases.

Bacteroids (genus Bacteroides).

Bacteroids are rod-shaped Gram-negative polymorphic bacteria that vary greatly in size. Obligate, non-spore-forming anaerobes. Can form capsules.

A typical representative *B. fragilis* is found in the folds of the mucosa at the base of the teeth, but is most typical for the intestine. *B. forsythus* is one of the periodontogenic species of microbes.

Porphyromonads (genus Porphyromonas)

Porphyromonads are short rod-shaped Gram-negative bacteria. Non-motile. Obligate non-spore-forming anaerobes. Dark pigmented colonies are formed on blood agar. The most common are *Porphyromonas asaccharolytica* (typical species), *P. endodontalis* and *R. gingivalis*. Their number increases in various purulent-inflammatory processes of the oral cavity: in dental granulomas, in purulent osteomyelitis of the jaws, in actinomycosis.

Prevotella (genus Prevotella)

Prevotella are Gram-negative polymorphic rods. Non-motile. Obligate non-spore-forming anaerobes, many of which form a dark pigment.

P. melaninogenica (typical species), *P. buccae*, *P. denticola*, *P. oralis*, *P. oris* are more common in the oral cavity. *Prevotella* inhabit the gingival groove, the pockets of the mucous membrane. They are involved in the occurrence of odontogenic infections in the oral cavity and the development of periodontal disease.

Fusobacteria (genus Fusobacterium)

Fusobacteria are Gram-negative polymorphic bacteria. They have the shape of thin spindle-shaped rods or polymorphic rods of different lengths with pointed ends. Non-motile. Obligate non-spore-forming anaerobes.

Fusobacteria are constantly present in the oral cavity (in 1 ml of saliva - tens of thousands). The pathogenicity of spindle-shaped rods increases sharply in mixed cultures with spirochetes, vibrios, anaerobic cocci. At various pathological processes their quantity sharply increases. Thus, in ulcerative-necrotic lesions (Vincent's angina, gingivitis, stomatitis) the number of fusobacteria increases by 1000–10000 times simultaneously with a sharp increase in the number of other anaerobic microorganisms, especially spirochetes. *Fusobacteria* are found in carious dentin and gingival pockets in periodontitis. The main lesions in humans are caused by *F. nucleatum* and *F. necrophorum*.

Leptotrichia (genus *Leptotrichia*)

Leptotrichia have the form of long threads of different thickness with pointed or swollen ends, give dense plexuses, can be arranged in pairs in the form of granular rods (Fig. 8). Leptotrichia are immobile, do not form spores and capsules. Obligate anaerobes. Leptotrichia ferment glucose to form large amounts of lactic acid, which lowers the pH to 4.5.



Fig. 8. Cells of *L. buccalis*. Gram stain

Leptotrichia (*Leptotrichia buccalis*) is present in the mouth constantly (often in the neck of the teeth) in large quantities (in 1 ml of saliva 10^3 – 10^4). In periodontal disease, the number of these bacteria in the oral cavity increases. *L. buccalis* acts as a center for the formation of plaque and tartar, as well as together with lactobacilli participates in the processes of demineralization of dental tissues.

Actinomycetes (genus *Actinomyces*)

Actinomycetes are Gram-positive rod-shaped or filamentous branched bacteria. When divided by fragmentation, they can form thin straight, slightly curved rods, often with thickenings at the ends, located singly, in pairs, in the form of letters "V, Y", or clusters resembling a front garden (Fig. 9). Non-motile. Obligate or facultative anaerobes.

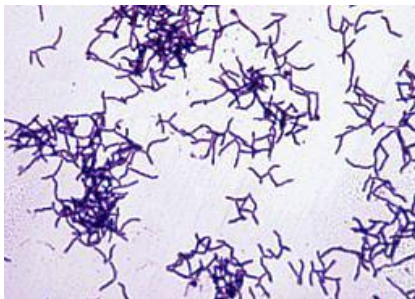


Fig. 9. Cells of *A. israelii*. Gram stain

Actinomycetes are almost always present in the mouth of a healthy person (*A. israelii*, *A. naeslundii*, *A. viscosus*, *A. odontolyticus*). Actinomycetes are involved in the development of caries, periodontal disease. When the resistance of the macroorganism decreases, actinomycetes can cause endogenous infection actinomycosis – a disease that occurs in the form of chronic purulent inflammation with the development of granulomas, abscesses and fistulas.

Spiral bacteria

Family Spirochaetaceae.

Spirochetes inhabit the oral cavity from the moment of eruption of baby teeth in a child and from that time become permanent residents of the oral cavity. They belong to three genera: *Borrelia*, *Treponema*, *Leptospira*. Very mobile. Active movements are carried out with the help of microfibrils that surround the bacterial cell.

The genus Borrelia is represented in the oral cavity by the following species: *B. Buccalis*, *B. vincentii*. *Borrelia* is a thick twisted short thread with 2–6 asymmetrical turns (*Fig. 10*). They do not form spores and capsules. According to Romanovsky-Gimza, they are stained blue-violet. Obligate anaerobes. Found in the folds of the mucous membrane and gingival pockets.



Fig. 10. *Borrelia* in dark-field microscope

Genus Treponema. *Treponema* have the form of a thin twisted thread with 8-14 uniform curls, close to each other (*Fig. 11*). According to Romanovsky-Gimza, they are painted in a pale pink color. Obligate anaerobes. *T. orale*, *T. macrodentium*, *T. denticola* are found in the oral cavity.

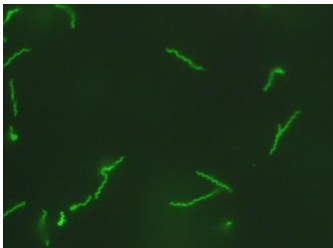


Fig. 11. Cells of *T. denticola*. Immunofluorescens microscopy

Genus Leptospira. Leptospira (*Leptospira dentium*) are thin spiral bacteria that form 15–30 small curls. The end parts of the leptospira are hooked in the form of letters C or S (*Fig. 12*). Spores and capsules are not formed. Aerobics or microaerophyls. According to Romanovsky-Gimza, they are painted pink.



Fig. 12. Leptospira. Dark-field microscopy

Spirochetes multiply intensively in the oral cavity with a significant reproduction of all anaerobic microorganisms. They cause pathological processes only in combination with other microbes, cocci, fusobacteria, vibrios. Many spirochetes are found in ulcerative-necrotic lesions of the mucous membrane (ulcerative stomatitis, Vincent's sore throat), in pathological gingival pockets, in severe forms of periodontitis, in carious foci and necrotized pulp.

Bacteria without cell wall

Family Mucoplasmataceae

Genus Mycoplasma. Mycoplasmas are small bacteria that do not have a cell wall, surrounded by a cytoplasmic membrane containing sterols. Due to the lack of cell wall, mycoplasmas have a variety of shapes: coccoid, filamentous, bulbous. Facultative anaerobes. Propagated by binary division, budding, fragmentation of filaments. *Mycoplasma orale* and *Mycoplasma salivarium* are present in the oral cavity. They are found in periodontal disease.

2. Oral fungi

Yeast-like fungi of the genus *Candida* are found in the oral cavity of healthy people in 40–50 % of cases. They have the form of oval or elongated cells, often with a branch of a new cell (*Fig. 13*). Pathogenic properties are most pronounced in *C. albicans*. In addition, other species of yeast-like fungi, such as *C. tropicalis* and *C. glabrata*, can be found in the oral cavity. They cause candidiasis in patients with immunodeficiency or long-term antibacterial therapy, which leads to dysbacteriosis. The clinical course may be in the form of local lesions of the oral cavity, or in the form of generalized candidiasis with multiple lesions of the internal organs.

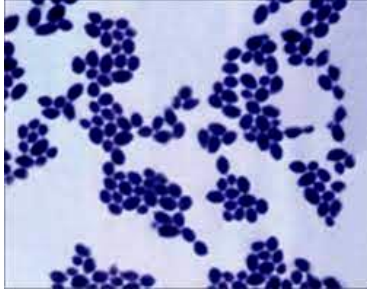


Fig. 13. Cells of *C. albicans*. Gram stain

3. Protozoa of the oral cavity

Entamoeba gingivalis, Trichomonas elongata (*T. tenax*) can grow in 50 % of healthy people in the oral cavity. Increased reproduction of protozoa occurs with unhygienic contents of the oral cavity. They are found mainly in plaque, crypts of the tonsils, in the purulent contents of periodontal pockets. In very large quantities, they are found in gingivitis and periodontitis.

Thus, half of the resident microflora of the oral cavity consists of streptococci, the other – of veilonella (about 25 %) and diphtheroides (about 25 %). All other microorganisms of the oral cavity – staphylococci, spirochetes, lactobacilli, fusobacteria, bacteroids, actinomycetes, neisseria, mycoplasmas, yeasts, protozoa are secondary representatives of the microflora and are in much less numbers. There are antagonistic or synergistic relations between these permanent representatives. It is believed that streptococci (*S. salivarius*, *S. sanguis*, *S. mitis*), veilonella and diphtheroids are a stabilizing part of the oral microflora, and streptococci (*S. mutans*), lactobacilli, bacteroids, actinomycetes are aggressive.

The number of microorganisms in the oral cavity varies during the day, with the leading role played by the production of saliva, which is sharply reduced at night. Factors that cause temporary or permanent changes in the content of individual members of the flora are antibiotics, changes in diet, physiological effects, elimination of all carious lesions of the teeth and removal of damaged teeth, various somatic diseases.

Characteristics of the main habitats of the oral cavity

The oral cavity, as an ecological niche, can be divided into several smaller biotopes, that are quite different from each other in the composition of the microflora:

1. The mucous membrane of the oral cavity.
2. Ducts of salivary glands with saliva.
3. Gum fluid and gingival area.
4. Oral fluid.
5. Dental plaque.

The mucous membrane of the oral cavity is the largest biotope in area and diverse in living conditions. Therefore, the microflora differs significantly in different parts of it.

On the surface of the mucous membrane mainly facultative anaerobic flora (mainly streptococci *S. oralis* and *S. sanguis*) vegetates. In the sublingual region, on the inner surface of the cheeks, in the folds and crypts of the oral mucosa, obligate-anaerobic species usually predominate: veilonella, peptostreptococci, lactobacilli, as well as streptococci *S. oralis*, *S. mitis*.

The tongue with its papillary surface provides places of colonization protected from mechanical removal. Streptococci (*S. salivarius* and *S. mitis*), veilonella, as well as peptostreptococci, actinomycetes and bacteroids are found here.

Various streptococci, corynebacteria, neisseria, hemophiliacs, pseudomonads, as well as yeast-like fungi and nocardias are found in large numbers on the mucous membrane of the hard and soft palate, palatine arches and tonsils.

The ducts of the salivary glands are one of the least studied habitats. It is believed that due to the high bactericidal activity of saliva, the ducts of the glands of a healthy person are almost sterile. However, the presence of a small number of bacteria, mainly veilonella is detected.

Gum fluid and gingival groove. This biotope is dominated by filamentous and spiral obligate anaerobic species of bacteria: fusobacteria, leptotrichia, actinomycetes and spirochetes. This is the main habitat of bacteroids. Protozoa, yeast-like fungi and mycoplasmas are also found here.

Oral fluid is the most important habitat of the oral cavity, because through it the interaction between all habitats of the oral cavity and the regulation of the microflora by the macroorganism are realised. Microbes that multiply on the mucous membrane of the oral cavity, in the gingival grooves, pockets, folds of the mucosa and in dental plaque constantly enter the oral fluid. In the oral fluid, they remain viable for a long time, and many species, especially those that do not have factors of adhesion to the mucosa and enamel, are actively reproducing. The oral fluid contains a significant amount of streptococci (*S. salivarius*), Neisseria, Veilonella. In addition, there are motile species – vibrios, spirilla and spirochetes.

Dental plaque is the most complex and multicomponent habitat that forms on the tooth surface. It is estimated that up to 90 % of all oral microflora is concentrated in plaque. Here are determined almost all representatives of the microbial flora of the oral cavity (dominated by streptococci, actinomycetes, lactobacilli). Individual features of the macroorganism (diet, lifestyle, occupational hazards, etc.) play an important role in the formation of this habitat.

In general, the species composition of individual parts of the oral cavity largely depends on the redox potential (RP) of the environment. The back of the tongue and the mucous membrane of the oral cavity are an aerobic environment, so in these habitats the growth of facultative anaerobes is supported better. Gingival cleft and interdental spaces have a negative RP, so in these areas obligate anaerobes are most actively reproducing.

Dynamics of formation of the microbiocenosis of the oral cavity

The formation of the microbiocenosis of the oral cavity is a multi-stage process of interaction of its various components. Colonization of the oral cavity by microbes depends on:

- the ability of microorganisms to adhere to various surfaces, primarily to the epithelium and enamel,
- the relationship of metabolism of different groups of microorganisms

To settle in the oral cavity, microorganisms must first attach to the surface of the mucous membrane or to the teeth. Adhesion is necessary to ensure resistance to saliva flow and subsequent colonization (reproduction). Adhesion is mediated by adhesins on the surface of bacteria and receptors of oral epitheliocytes, structures of tooth enamel. In the process of adhesion by Gram-negative bacteria pili or fimbriae may be involved, while in Gram-positive bacteria lipoteichoic acid may be as adhesins. On the other hand, specific receptors of oral epitheliocytes are involved in the adhesion process (specific interactions are also present during adhesion to the tooth surface). Some bacteria do not have their own adhesins, then they are fixed on the surface of the mucous membranes, using the adhesins of other microorganisms, so there is a process of coaggregation between bacterial species of the oral cavity.

Streptococci of different species co-aggregate with actinomycetes, *F. nucleatum*, *Veillonella*, *Haemophilus parainfluenzae*. *F. nucleatum* binds to *Porphyromonas gingivalis*, *Haemophilus parainfluenzae* and *Treponema* spp. Coaggregation is an example of commensalism and synergism that arise between microbial species. It makes possible the indirect adhesion of some bacteria to epitheliocytes and tooth surfaces and may be important in the development of dental plaque because it promotes the colonization of bacteria that are unable to adhere to the pellicle. Another example of coaggregation is the synthesis by *S. mutans* extracellular polysaccharides from sucrose. These polysaccharides promote the attachment of bacteria to the teeth and the stability of the plaque matrix.

Relationships in the microbial community of the oral cavity can be mutually beneficial or antagonistic and aimed at maintaining homeostasis of the oral flora. The microflora of the oral cavity is significantly affected by the presence of food substrates, vitamins, RP, pH, the release of inhibitors that act on reproduction. Different species of bacteria cooperate in the use of substrates that they are unable to metabolize alone. Thus, *Fusobacterium nucleatum* and *Porphyromonas gingivalis* synergistically hydrolyze casein.

The development of complex food chains also contributes to the diversity and stability of ecosystems. In the presence of sucrose that comes with food, there is a rapid development of microaerophilic streptococci *S. mutans* and *S. sanguis*, as well as lactobacilli. Lactic and formic acids that are synthesized by

them and some other anaerobic bacteria are an energy source for veilonella. Corynebacteria synthesize vitamin K which is the most important growth factor of bacteroids, peptostreptococci, fusobacteria and veilonella. Yeast and yeast-like fungi synthesize B vitamins, which stimulate the growth of various members of the oral cavity.

The use of oxygen by facultative anaerobes reduces the concentration of O₂ and RP to levels suitable for colonization of mucous membranes by strict anaerobes.

The normal composition of microorganisms in this ecological niche is maintained largely due to the antagonistic relationship between microbes. Thus, microaerophilic streptococci are antagonists of fuso- and corynebacteria due to the production of acidic metabolites, hydrogen peroxide, various bacteriocins. Veilonella, utilizing organic acids, dramatically increase the pH of the environment, which in turn inhibits the development of cariogenic flora – streptococci and lactobacilli. Leptotrichia, bifidobacteria and lactobacilli, sharply acidifying the environment, are antagonists of yeast and yeast-like fungi, which leads to a decrease in vitamin synthesis and inhibition of the growth of many microorganisms.

Age-related changes of the microflora

Normally, the fetus is in gnotobiological conditions, ie sterile. The first germs begin to appear in the body of the child during the birth. From this moment so-called primary microbial colonization of the human body begins. In the first 6–8 hours after birth there is a rapid increase in the number of bacteria in the oral cavity. During this period, the child's oral cavity is colonized by aerobic and facultative anaerobic species: diphtheroids, Neisseria, sarcins, lactobacilli, streptococci. There are no obligate anaerobic species.

The maximum diversity of the microflora reaches till 2–4 months of life. During this period, a significant number of lactobacilli, bifidobacteria, Neisseria, hemophilic bacilli, microaerophilic streptococci, especially *S. salivarius*, as well as yeast and yeast-like fungi are found in the oral cavity. In the folds and lacunae of the mucous membrane obligate anaerobes appear such as veilonella and some fusobacteria.

With the advent of teeth, conditions are created for the growth of obligate anaerobic species and bacteria with high adhesive properties to enamel. Thus, there are microaerophilic streptococci *S. mutans* and *S. sanguis*, actinomycetes. In preschool children, the microflora of the oral mucosa and gingival gutter already resembles the microflora of adults and contains leptotrichia, bifidobacteria, peptostreptococci, fusobacteria and spirila. Most children do not normally have bacteroids, spirochetes and protozoa.

During puberty, almost all types of microorganisms characteristic of the adult organism are identified as part of the microbiocenosis. Against the background of changes in the hormonal background bacteroids, protozoa and spirochetes appear.

Loss of teeth in old age leads to a significant reduction in the content of obligate anaerobes.

MICROBIOLOGY OF CARIES AND RESEARCH OF MICROFLORA IN CARIES

Dental plaque, the mechanism of its formation, localization. Adhesion and coaggregation of bacteria

Caries is a pathological process in which there is demineralization and softening of the hard tissues of the tooth, followed by the formation of a cavity.

The key mechanism of occurrence and development of dental caries and periodontal disease is the formation of dental plaque. Dental plaque is an accumulation of bacteria in the matrix of organic matter, mainly proteins and polysaccharides brought there by saliva, which are produced by the microorganisms themselves. Plaques are tightly attached to the surface of the teeth. Dental plaque is usually the result of structural changes in plaque, consisting of microorganisms with little inclusion of unstructured matter of organic nature and having a porous structure. The accumulation in the plaque of the final products of life of microorganisms and mineral salts slows down the diffusion of saliva and liquid components of food inside, as the porosity of plaque disappears. As a result, there is a new formation or dental plaque, which can be removed only by force and not completely.

There are several mechanisms in the formation of plaque:

1. Deposition of salivary glycoproteins that form a pellicle with subsequent specific adhesion of bacteria to it.
2. Adhesion of epithelial cells infested with bacteria to the enamel, with subsequent growth of microcolonies.
3. Precipitation of extracellular glycans produced by *S. mutans* and *S. sanguis*.
4. Agglutination of bacteria with antibodies, followed by fixation on the enamel surface. It is known that bacteria in dental plaque are coated with class A and G immunoglobulins.

The process of plaque formation begins after brushing the teeth with the formation of a film on the surface of the tooth that is a pellicle. The main components of this film are components of saliva and gingival fluid, such as proteins (albumins, lysozyme), glycoproteins (lactoferrin, IgA, IgG, amylase), phosphoproteins and lipids. Bacteria colonize the pellicle for the first 2–4 hours after cleaning. During this period, the bacteria are weakly bound to the film and can be quickly separated by the flow of saliva. After primary colonization, the most active species begin to grow rapidly, forming microcolonies that penetrate the extracellular matrix. Then the process of bacterial aggregation begins and at this stage the components of saliva are connected. The first microbial cells settle in the depressions on the tooth surface, where they multiply, after which they first fill all the depressions, and then move to the smooth surface of the

tooth. Many microbial cells are unable to attach directly to the enamel, but can settle on the surface of other bacteria already adhered, ie there is a process of coaggregation. An example of coaggregation is the synthesis of *S. mutans* extracellular polysaccharides of glycans from sucrose. These polysaccharides help attach bacteria to tooth enamel and stabilize plaque matrix.

The adhesion process is very fast: after 5 minutes the number of bacterial cells per 1 cm² increases from 10³ to 10⁵–10⁶. In the future, the adhesion rate decreases and is stable for approximately 8 hours after application. After 1-2 days, the number of attached bacteria increases again, reaching a concentration of 10⁷–10⁸. Thus, plaque is formed. Then there are its structural changes and the formation of dental plaque.

If we talk about the change in the microbial population, the primary bacteria that attach to tooth enamel are streptococci (*S. mutans* and *S. sanguis*). In addition, *Neisseria*, *veilonella*, diphtheroids take part in the formation of "early" dental plaque (the first 1–4 hours). Then the so-called dynamic plaque is formed (up to 4–5 days). At this stage, there is a decrease in the number of Gram-positive cocci and an increase in Gram-negative rods (*leptotrichia*, *fusobacteria*) and cocci (*veilonella*). On day 6–7, a mature dental plaque is formed. It is dominated by anaerobic rods. Such plaque can remain balanced for a long time.

Thus, the formation of plaques is initially dominated by aerobic and facultative anaerobic microflora, which sharply reduces the redox potential in this place, thus creating conditions for the development of strict anaerobes.

There are supra- and subgingival plaques. The first have pathogenetic significance in the development of dental caries, the second – in the development of pathological processes in the periodontium. The microflora of plaques on the teeth of the upper and lower jaws differs in composition: the plaques of the teeth of the upper jaw are more often inhabited by streptococci and lactobacilli, on the plaques of the lower jaws - *veilonella* and filamentous bacteria. Actinomycetes are isolated from plaques on both jaws in equal quantities. It is possible that this distribution of the microflora is due to different pH values of the medium.

The concept of biofilms

Currently, scientists consider plaque as a biofilm. Biofilms (biological films) are organized communities of microbes that form in liquid environments.

The main properties of the biofilm are: 1) interacts with different types of microorganisms (microbiocenosis) with symbiotic relationships; 2) microorganisms form microcolonies surrounded by a protective matrix permeated by channels through which nutrients, products of life, enzymes, metabolites and oxygen circulate; 3) microorganisms have a certain communication system due to the production of special substances of autoinducers, which provide self-regulation of the microbial community; 4) microorganisms in biofilms are resistant to antibiotics, antimicrobials and reactions of the host organism.

Studies conducted on biofilms in their natural state have shown that there are large differences in the behavior of bacteria in laboratory culture and in their natural ecosystems. For example, a bacterium in biofilms produces substances that it does not produce in planktonic culture. In addition, the matrix around the microcolonies serves as a protective barrier. This helps to understand why antimicrobials, both general and local, do not always give successful results, even when they target a specific type of microorganism.

Pathogenesis of caries

Currently, the generally accepted mechanism of caries is the progressive demineralization of the hard tissues of the teeth under the action of organic acids, the formation of which is associated with the activity of microorganisms.

Normally, tooth enamel is in a state of dynamic equilibrium between the ongoing processes of de- and remineralization. Demineralization is caused by free hydrogen ions H^+ , the main source of which are organic acids - products of metabolism of oral microorganisms. The rate of destruction of enamel significantly increases when the pH value of the medium falls below 5. Of great importance in the development of the carious process is the duration of contact of acidic products with tooth enamel.

Caries develops on those surfaces of the tooth that are in prolonged contact with the formed acids. This leads to a gradual increase in the microspace between the crystals of enamel prisms. Microorganisms penetrate into the formed small defects and damage enamel on the sites located parallel to an external and internal surface. The long process of demineralization ends with the dissolution of a stable surface layer and the formation of a cavity in the tooth.

The main condition for the development of caries is the formation of dental plaque, due to which the local demineralizing action of the microbial flora that inhabits it (lactic acid production as a result of glycolysis) is caused. The development of caries is facilitated by the entry into the oral cavity of carbohydrates, which are food substrates for microorganisms and the starting material for the synthesis of organic acids.

Cariogenic microorganisms

Oral streptococci (*S. mutans*, *S. sanguis*), lactobacilli and some actinomycetes are of the greatest importance in the development of caries.

The leading role is played by *S. mutans*, consisting of 8 serovars. It is the most acid-forming representative among oral streptococci and can exist at low pH values. One of the most important biological properties of *S. mutans* is the ability of these bacteria to attach to the smooth surfaces of the tooth. Adhesion to the teeth ensures the formation of plaque by these microbes. Streptococci ferment many carbohydrates to form lactic acid. The pH in the plaques decreases to a critical level (pH 5 and below). Along with acid formation, the ability of oral streptococci to form extracellular polysaccharides such as soluble and insoluble glycans (dextran) and levan (fructan) is of pathogenetic importance.

Soluble glycans and levan are easily cleaved by both *S. mutans* and other microorganisms, and insoluble glycan is actively involved in the adhesion of oral microorganisms. Glycan formation causes intercellular aggregation of *S. mutans* and other bacteria present in the plaque (*Nocardia*, *Neisseria*, *A. viscosus*, *C. albicans*). Glycans stabilize the plaque. The sticky glycan matrix of dental plaque prevents the diffusion of large amounts of lactic acid, which is formed by microbes, which prolongs its stay on the tooth surface and leads to demineralization of enamel, causing tooth decay. In addition, extracellular polysaccharides, filling the entire volume of the plaque or lesion, complicate the remineralization process, preventing the entry of calcium and phosphate ions into the enamel.

If streptococci predominate in the oral cavity, the number of lactobacilli in the plaque is approximately 1 % of the total number of microbes in the plaque. Lactobacilli play a minor role in the initial stages of adhesion of microbes to tooth enamel and in the formation of plaque. However, their role increases sharply in the progression of caries with increasing severity of carious lesions. Lactobacilli are tolerant to low pH and are able to synthesize large amounts of lactic acid from carbohydrates. Obviously, these microbes play a crucial role in the destruction of dentin after enamel deformation. As for the role of actinomycetes in the occurrence of caries, they probably participate in carious lesions of the roots of the teeth in the elderly when exposing the root of the tooth. In addition, actinomycetes, bacteroids and other microorganisms secrete proteases that are involved in the destruction of dentin, and therefore increase carious lesions.

The cariogenic activity of oral microorganisms is influenced by saliva, its aggregating factors, which, on the one hand, contribute to the attachment of microbial cells to the tooth surface, and on the other – remove them when washing the mouth. The balance between the processes of de- and remineralization is influenced by many factors such as the presence in the saliva of bicarbonate, urea, calcium ions, phosphorus and others. When the pH drops below the critical level (5 or less), calcium and phosphorus ions are released from tooth enamel into the environment. With increasing pH, they are part of the enamel back. A buffer system of bicarbonate-carboxylic acid and sialin in saliva has the ability to increase the pH value and, therefore, anti-cariogenic effect.

Algorithm: *"Microbiological methods for examination of the microflora in caries and its complications."*

Studies of the microflora in caries are carried out using bacterioscopic and bacteriological methods. The material for the study can be dental plaque, material from the carious cavity, oral fluid.

Examination of dental plaque

Before removing plaque, it is necessary to carry out a thorough hygienic treatment of the oral cavity, using various mechanical methods and controlling the treatment by determining the index of hygiene. For this purpose, use special staining solutions to determine the area of plaque.

At research of a dental plaque it is necessary to consider:

- the method of removing plaque from the tooth surface;
- method of dispersion of plaque material;
- methods of microscopic counting and counting the survival of microbes in cultivation.

Method of taking plaque material

The plaque, located on the accessible smooth surface of the tooth (from the cheeks, tongue), can be removed by scraping with a conventional sterile instrument: excavator, scaler. Sterile thread can be used to remove plaque from the proximal surfaces. Plaque from pits, fissures can be obtained with a sharp probe or pointed orthodontic wire. In some cases, the material is taken with small sterile cotton swabs. However, due to the density of adhesion of the plaque and the difficulty of its removal, this method is only suitable for studying the initial stage of colonization of microbes on the enamel. The supragingival dental plaque can be removed with a sterile excavator or scaler.

For bacteriological examination, the material must be placed in a transport medium immediately after collection in order to preserve the viability of microbes. The obtained material is weighed on analytical scales with subsequent dilution from 1 : 100 to 1 : 1000 and seeding on nutrient media.

Method of dispersion of plaque material

The accuracy of determining the number and types of bacteria in the plaque depends on the thoroughness of the material dispersion.

You can break the conglomerates of plaque by shaking with glass beads in a homogenizer, processing the material in ultrasonic disintegrators. However, ultrasound can kill some bacteria: spirochetes and some Gram-negative bacteria are particularly sensitive to ultrasound. In this regard, ultrasound treatment is usually carried out for 10 seconds.

Microscopic calculation of microbial survival during cultivation

Direct microscopic counting suspended microbes can be performed in Goryaev's chamber (a device designed to count the number of cells in a given volume of fluid) (*Fig. 14*).

The count of viable cells from the sample is performed by serial dilutions in sterile saline (1 : 10, 1 : 20, and so on). From each dilution, a certain volume is seeded on the surface of a solid medium. After incubation, the quantities of CFU (colony-forming units) are counted and the quantity is converted to the original volume.

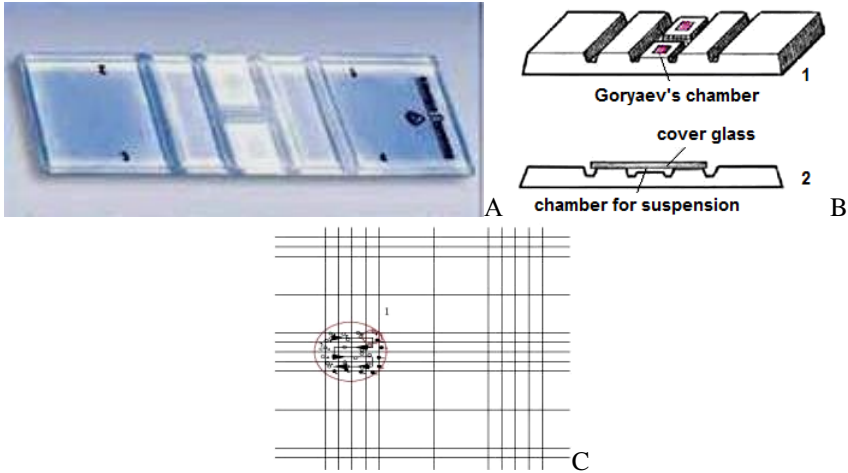


Fig. 14. Goryaev's chamber:

A – chamber; B – scheme: 1 – top view, 2 – side view; C – a net

Collection of material from the carious cavity

First, the surface layers of softened dentin soaked in saliva are removed from the carious cavity with sterile boron. Without allowing saliva to enter the test material, the cavity is treated with another sterile boron and dentin is placed with a sterile trowel in the transport nutrient medium.

Oral fluid intake

Oral fluid is collected from patients in the morning (9–11 hours) 2 hours after a meal for 10 minutes in sterile tubes. This saliva is called unstimulated. Stimulated saliva is obtained after applying to the back of the tongue 1–2 drops of sterile 2 % citric acid solution or chewing 5 mg of paraffin for 30 seconds. Parotid saliva is obtained by inserting a special sterile cannula into the duct. The oral fluid is collected in a sterile test tube, 0.1 ml is examined.

MICROBIOLOGY OF PERIODONTAL DISEASES

Periodontal microflora in the absence of pathologies

The tissues of a healthy periodontium are associated with a rather limited flora located under the gums on the tooth surface. Periodontal microbes form a layer with a thickness of 1 to 20 cells. Examination in the area of the gingival groove revealed a rather thin layer (about 60 nm), consisting of 3/4 of Gram-positive cocci. Together with rods, they make up 90 % of the population. Spirochetes are rare – about 1.8 %. The ratio of motile to immobile forms in healthy tissues is 1/49.

In the gingival grooves, plaques consist mainly of Gram-positive facultative anaerobic cocci (streptococci, fewer – staphylococci, peptostreptococci) and rods (actinomycetes: *A. israelii*, *A. naeslundii*, *A. viscosus*, *A. odontolyticus*, and also Propionibacteria).

Periodontal disease. Definition, classification

Periodontal diseases are heterogeneous groups of diseases of inflammatory and metabolic-dystrophic nature, accompanied by destruction of gum tissue, including the collagen base of the periodontium and the bones of the alveolar ridge.

Periodontal diseases include:

1. Gingivitis (local inflammation of the gums).
2. Parodontitis (progressive inflammatory process with destruction of periodontal and bone tissues).
3. Periodontosis (mainly dystrophic lesions of periodontal tissues).
4. Periodontomes (tumor and tumor-like processes of periodontal tissues).

Inflammatory periodontal diseases (gingivitis and periodontitis) are widespread among the population (60–70 % after 30 years and 85–97 % after 65 years) and are the leading cause of tooth loss in most adults.

Periodontopathogenic microorganisms

In the pathogenesis of inflammatory periodontal diseases there is an interaction of two pathogenetic mechanisms: the influence of anaerobic microflora and immunological reactivity of the human body.

Periodontopathogenic species of bacteria differ from others by high adhesive, invasive and toxic properties in relation to periodontal tissues. They include:

1. Gram-negative anaerobic bacteria of the group of bacteroids (*Prevotella melaninogenica*, *Porphyromonas gingivalis*, *Tannerella forsythensis*), less often - spirochetes and fusobacteria.
2. Gram-positive anaerobic bacteria of the actinomycete group, less often - peptostreptococci.

Periodontopathogenic microorganisms have a wide range of pathogenic factors, which allows them to induce a prolonged inflammatory process. They include:

- Adhesion factors – the ability to adhere in large quantities to epithelial cells, hydroxyapatite and Gram-positive bacteria. Their adhesive properties are inhibited in the presence of saliva and blood serum. However, the ability to coaggregate with Gram-positive bacteria is not reduced.
- Invasion factors – production of histolytic enzymes: hyaluronidase, DNAase, RNAase, collagenase, protease.
- Toxic factors – Gram-negative bacteria contain endotoxin, produce cytotoxic substances such as fatty acids, indole, amines, ammonia and others. All these substances have a destructive effect on periodontal tissues. Bacteroids secrete volatile sulfur compounds that increase the permeability of the oral

mucosa. In addition, due to the content of specific lipopolysaccharides, Gram-negative microbes can cause immunopathological mechanisms that lead to the destruction of bone tissue. Some types of bacteroids, fusobacteria and others have such properties in vitro.

- Protective properties, ie the ability to counteract the protective forces of the macroorganism. This property is provided by a polysaccharide capsule of Gram-negative microbes, enzymes capable of breaking down immunoglobulins and complement fractions.

Many types of bacteroids are able to produce enzymes that inactivate antibiotics, which complicates treatment.

Microflora in gingivitis

The total number of microbes in gingivitis is 10–20 times greater than in a healthy periodontium. Even before the onset of clinical symptoms, microscopy can detect an increase in Gram-negative flora. In this sense, the preclinical phase of inflammatory periodontal disease can be considered as a kind of dysbacteriosis, which leads to improper lifestyle, metabolic disorders in periodontal tissues, endocrine dysfunction.

With prolonged gingivitis, the subgingival flora is characterized by an increase in the number of Gram-negative rods: fusobacteria, bacteroids, etc., which make up about 45 % of all cultivated flora. Gram-positive facultative anaerobic rods, mainly *Actinomyces naeslundii*, *A. viscosus*, *A. israelii* are detected with a frequency of about 25 %. Propionibacteria and eubacteria are released in small amount. In 27 % of cases, Gram-positive facultative anaerobic streptococci are detected.

Microflora in parodontitis

With parodontitis, there is a significant shift towards rod-shaped forms and spirochetes, the number of which increases to 40 %. The ratio of motile to non-motile forms increases to 1 : 1 (normally 1 : 49). Mainly Gram-positive microbes are attached to the cement, Gram-negative are present in the loose layers of the subgingival plaque, which spreads to the top of the pocket.

In parodontitis, Gram-negative anaerobic rods (*Porphyromonas gingivalis*, *Prevotella melaninogenica*, *Fusobacterium nucleatum*, etc.) predominate. However, in some patients there is a predominance of actinomycetes.

Mechanism and conditions of periodontal disease

In the pathogenesis of periodontal disease a significant role belongs to microbial factors and immunopathological mechanisms – immunocomplex and cellular.

For the development of periodontal disease, the following conditions must be combined:

1. The presence of periodontopathogenic species of bacteria in sufficient quantities to begin the pathological process.

2. Living conditions in the niche should promote the growth and reproduction of bacteria (sufficient nutrients, growth factors, low RP).

3. Periodontal tissues should be free of microbial antagonists of periodontal pathogenic bacteria.

4. The microbe must be spatially localized so that it or the products of its vital activity can act on the target cells.

5. The human body must be sensitive to microbes or their products.

6. Development of immunopathological reactions. When examining the contents of the gingival pocket in patients with periodontitis, immunoglobulins of classes A, G, M, complement fractions C3, C5, leukocytes are determined. Gum tissues are abundantly infiltrated by plasma cells, lymphocytes, macrophages (monocytes). All this suggests that many antigen-antibody reactions, manifestations of cellular immunity occur here, in the tissues of the periodontium and alveolar bone.

Immunopathogenesis of periodontal diseases can be divided into two phases: reversible and irreversible.

The reversible phase is associated with a normal protective immune response from local tissues. Its mechanism is due to the increased reproduction of Gram-negative bacteria in the gingival pockets and dental plaques. Microbial enzymes loosen the barrier-impermeable barrier, the marginal epithelium of the gums, and create the conditions for the transfusion of endotoxins into connective tissue. Microbial antigens, cell breakdown products and metabolic products of dental plaque provoke increased migration of segmental leukocytes and macrophages in the marginal epithelium. As specific antibodies (IgM, IgG) accumulate, they form immune complexes with persistent antigens of microbial nature, which should help clear the oral mucosa from them. Capture and degradation of immune complexes and their breakdown products are carried out by phagocytes migrating to the inflammatory center, activated by lymphokines.

The reversible phase is clinically manifested by signs of local inflammation ie gingivitis. Timely treatment stops the massive influx of antigens and stops or eliminates gingivitis. However, if the massive influx of microbial antigens does not stop, the mobilized defense mechanisms can lead to tissue destruction. This is due to the release of phagocytic cells of lysosomal enzymes, among which the most active proteinases such as collagenase and elastase. They are able to break down denatured collagen of periodontal connective and bone tissues. The epithelium swells, loses a strong connection with the hard tissues of the tooth. As a result, a pathological gingival pocket is formed, which serves as a gateway for secondary purulent infection. In this case, gingivitis turns into periodontitis.

Irreversible, immunopathological, phase, first of all, is connected with sensitization of T-lymphocytes by the autoantigens formed at destruction of a periodontium. An important role is played by microbial endotoxins, which enhance the sensitization of lymphocytes, and can also cause polyclonal activation of B-lymphocytes. Thus, the mechanisms of autoaggression are

formed, leading to a progressive, recurrent, irreversible course of parodontitis with atrophy of osteocytes and alveolar processes of the jaw.

Understanding the etiology and pathogenesis of parodontitis is necessary not only to establish the role of microbes in this process, but also to clarify the conditions that promote plaque growth, determine the role of local and systemic factors that may affect the resistance or sensitivity of periodontal tissues to bacteria, products their vital functions and the importance of individual characteristics of the host organism in the functioning destructive and protective mechanisms.

Algorithm: "Methods of examination of the microflora in periodontal disease"

Bacterioscopic and bacteriological methods are used in the diagnosis of periodontal diseases. The molecular biological method (PCR) is currently being actively developed. Existing diagnostic kits allow to identify 5 main periodontopathogenic species of microorganisms. The material for the study may be gingival fluid, subgingival plaque.

Gum fluid intake.

Gingival fluid can be taken with a small sterile curettage spoon, a scaler from the gingival groove, pathological gingival pocket. Gum fluid can be collected on the principle of capillarity with a sterile micropipette, sterile filter strips, sterile threads.

Collection of material from the gingival pocket for bacterioscopic examination.

In the gingival pocket, some bacteria are in a fixed state on the surface of the root (dental plaque), and others – in a free state in the gingival fluid. Therefore, the material can be taken on celluloid narrow plates, which are carefully inserted into the pocket and pressed to the root surface from the gums. On the inside of the plate, microbes stick to the root of the tooth, and on the outside, germs stick freely in the gingival fluid.

From the removed teeth you can make scrapes or make histological sections.

Collection of material from the gingival pocket for bacteriological examination.

Subgingival dental plaque from the periodontal pocket can be obtained with a sharp probe, orthodontic pointed wire. It must be disintegrated before seeding, because the accuracy of determining the number and types of bacteria in the plaque depends on the thoroughness of the material dispersion.

For bacteriological examination, the material must be placed in a transport medium immediately after collection in order to preserve the viability of microbes. Further isolation of pure cultures, their cultivation and identification are carried out in parallel in anaerobic and aerobic conditions according to the classical scheme.

PURULENT-INFLAMMATORY PROCESSES OF THE ORAL CAVITY

Odontogenic infection of the maxillofacial region

Odontogenic process is an inflammatory process that is directly related to the tissues inside and around the tooth. The development of odontogenic inflammatory processes is determined by the peculiarities of the anatomical and topographic relationships between the entrance gate of infection that is odontogenic focus and the surrounding tissues: periosteum, bone and soft tissues of the maxillofacial region. Anatomical proximity, a large number of blood and lymphatic vessels connecting these tissues, create favorable conditions for the rapid spread of infection.

The carious process makes it possible for microbes to enter the pulp through the dentinal tubules. Further spread of microbes and products of their vital activity causes the development of periodontitis, and then the inflammatory process spreads to the periosteum, and there is periostitis, osteomyelitis. Involvement of soft tissues in the inflammatory process leads to maxillary abscesses and phlegmon.

Pulpitis is an acute or chronic inflammatory process that occurs in the coronal or root pulp.

Periodontitis is an inflammation of the periodontium, characterized by a violation of the integrity of the ligaments that hold the tooth in the alveoli, the cortical plate of the bone that surrounds the tooth and the resorption of bone tissue from small size to the formation of large cysts.

Periostitis is an inflammation of the periosteum.

Osteomyelitis is a purulent-necrotic process that develops in the bones and bone marrow.

Abscess is a purulent inflammation of tissues with their melting and the formation of a purulent cavity, which can develop in the subcutaneous tissue, muscles, bones (localized inflammatory process).

Phlegmon is an acute diffuse purulent inflammation that spreads to several areas of the head and neck.

An important feature of these forms of odontogenic inflammation is the spread of inflammation in adipose tissue along the fascia, therefore, the term fasciitis is accepted. Progression of purulent inflammation can lead to hematogenous (with blood flow) spread of infection that is sepsis.

Microbial flora in pulpitis

Healthy pulp is a biological barrier that prevents the penetration of various harmful factors into the periodontal tissues. Acute pulpitis is initially focal in nature and proceeds as a serous inflammation. Most often, greenish and non-hemolytic group D streptococci, streptococci without group antigen, lactobacilli are detected. Without treatment, acute serous pulpitis turns into purulent pulpitis, in which peptostreptococci, beta-hemolytic streptococci of groups F and G are isolated.

Acute pulpitis turns into chronic, and with tissue necrosis into gangrenous pulpitis. In these forms of pulpitis, anaerobic bacteria are isolated in large quantities from necrotized pulp: peptostreptococci, beta-hemolytic streptococci of groups F and G, bacteroids, spirochetes, actinomycetes. Putrefactive bacteria can also join such as *Proteus*, clostridia, bacilli.

Microbial flora in periodontitis

Depending on where the microbes get into the periodontal tissues, there are apical periodontitis (inflow through the root canal) and marginal (penetration from the pathological gingival pocket).

In acute serous periodontitis, the microflora often includes greenish and non-hemolytic streptococci. If the inflammation is associated with the penetration of microbes through the opening of the root canal, the microbial composition is determined by the flora of purulent pulpitis.

In purulent periodontitis, coagulase-positive *S. aureus* and β -hemolytic streptococci are detected.

In the transition from acute to chronic periodontitis, anaerobic peptostreptococci begin to predominate, which are joined by other streptococci with group and without group antigen. Actinomycetes, bacteroids, fusobacteria, spiral bacteria, and clostridia are found in apical granulomas. Odontogenic infections have been shown to be more severe if anaerobes, especially *F. nucleatum*, are involved in their genesis.

Microbial flora in periostitis, osteomyelitis, abscesses, phlegmons of the maxillofacial area

In all the above diseases, microbial associations are found, which are dominated by anaerobic Gram-positive and Gram-negative rods, peptostreptococci, staphylococci (*S. aureus*), β -hemolytic streptococci, putrefactive bacteria.

The role of normal microflora in the development of inflammatory processes of the maxillofacial area

Inflammatory diseases caused by opportunistic microbes affect any tissues of the maxillofacial area: mucous membranes, adipose tissue, muscles and fascia, ligaments and bones. Most of the infections discussed above are endogenous.

One thing is that anaerobes are the majority in the microbial biocenosis of the oral cavity, suggests their main role in the development of pathological processes of the maxillofacial area. Non-spore-forming anaerobic bacteria are representatives of the oral microflora that cause odontogenic purulent-inflammatory processes include representatives of the genera *Bacteroides*, *Porphyromonas*, *Prevotella*, *Fusobacterium*, *Leptotrichia*, *Peptococcus*, *Peptostreptococcus*, *Actinomyces*.

Being opportunistic pathogens, they have a number of advantages:

- High levels of their content in the oral cavity, resulting in a high probability of endogenous infection.
- Resistance to most antibacterial drugs.
- Presence of virulence factors:

1. Surface structures of the cell (pili, capsule).
2. Enzymes (collagenase, neuraminidase, DNAase, heparinase, fibrinolysin, beta-lactamase).
3. Toxins (endotoxin, leukocidin, hemolysins, hemagglutinin).
4. Metabolites (volatile and long-chain fatty acids).

Associations of 3–5 or more species of obligate anaerobic bacteria or their combination with facultative anaerobes (more often with staphylococcus and streptococcus) and aerobes (*Neisseria*, *Pseudomonas aeruginosa*) can be determined simultaneously in the material from the patient. Symbiotic relationships that develop between different bacteria of the oral mucosa or dental plaque, with the development of the infectious process provide synergism of their pathogenic action in the inflammatory focus.

Bacteroids are most often isolated from purulent foci, and in 1/3 of patients prevail as a part of microbic association. Peptostreptococci predominate in 20 % and staphylococci in %. It should be noted that this pattern can be traced regardless of the prevalence of purulent-inflammatory process, but depends on its duration and treatment. The clinical picture does not depend on the type of pathogen, but on the affected organ.

Conditions for the development of odontogenic inflammation:

1. The microflora goes beyond its inherent ecological niche.
2. Reduction of the body's natural resistance (in particular, antimicrobial immunity).
3. The presence of conditions for the reproduction of anaerobic microflora (low RP environment, the presence of growth factors).

Conditions for the microflora to go beyond its inherent ecological niche in the body can be local, mechanical, or general, associated with a violation of regulation and resistance of the organism. Local conditions include: trauma to the oral mucosa, tooth extraction, other surgical interventions, tissue necrosis, punctures, endoscopy, tumor disintegration, etc.

Decreased natural resistance of the body may be associated with blood loss, starvation, hypothermia, fatigue, local circulatory disorders. Recently, this list has been supplemented by adverse effects on the immune system of surgery, injuries and burns, the use of immunosuppressants, cytostatics, antibiotics and glucocorticoids. Surgical infections are now increasingly occurring against the background of diseases of malignant tumors, diabetes, radiation therapy, leukopenia, hypogammaglobulinemia, in the condition after organ transplantation. All of the above reasons, violating antimicrobial immunity, contribute to the development of endogenous infections, especially in medical institutions, where the patient is protected from external (transient) microflora by aseptic hospital treatment.

Negative redox potential of the environment and the presence of growth factors are required as the main conditions for the development of non-spore anaerobes at the site of penetration into the tissue. These conditions can exist

before the microorganisms enter the tissues (for example, in diabetes, the partial pressure of oxygen in the muscles and subcutaneous tissue is 40 % below normal; low pressure is observed in dead tissue, ischemia, vasospasm or compression), and can be created during the invasion itself.

A special place in the pathogenesis of infections caused by anaerobes, is their symbiosis with each other or anaerobes with aerobic microorganisms. This is mainly due to the absorption of free oxygen in the tissues by aerobes and the fact that aerobes synthesize special substances (growth factors) that promote the growth of anaerobes.

The presence of chronic inflammatory foci in the oral cavity is of great importance in the pathogenesis of odontogenic infections. Chronic localized processes in the oral cavity in some cases can cause systemic diseases and are called chronic foci of infection. Chronic odontogenic foci of infection in the oral cavity are chronic gangrenous pulpitis, chronic periodontitis, parodontitis, chronic pericoronitis, chronic osteomyelitis.

Features of the composition of the microflora in odontogenic and neodontogenic inflammatory processes of the maxillofacial area

Acute odontogenic inflammatory diseases are characterized by the presence of non-spore-forming anaerobic bacteria (bacteroids, fusobacteria, peptococci, peptostreptococci, rarely actinomycetes), as well as streptococci.

In chronic odontogenic inflammations associations of obligate anaerobic bacteria with facultative anaerobes (staphylococci, streptococci) are isolated.

Neodontogenic inflammatory processes are characterized by a predominance of staphylococci, streptococci, rarely bacilli and obligate anaerobes. At the same time, monocultures are often isolated.

Methods of studying the microflora in odontogenic diseases

In odontogenic inflammatory processes (periodontitis, abscesses, phlegmons, etc.) microbiological methods are used not only to determine the etiology and pathogenesis of the disease, but also to control treatment, predict the outcome of the disease and determine the sensitivity of the microbial association to antibiotics.

The leading role in the development of inflammatory diseases of the oral cavity and maxillofacial area is played by obligate anaerobic and microaerophilic bacteria, which determines the need to create anaerobic conditions in the collection of material and cultivation of bacteria.

The material for the study of odontogenic inflammatory processes is the material from the root canal (for pulpitis), punctures, pieces of tissue, purulent discharge.

The main method of studying the microflora in odontogenic inflammation of the maxillofacial area is the bacteriological method.

In this pathology, the bacteriological method of research is usually a parallel step-by-step study of the material under aerobic and anaerobic conditions. Anaerobic jar and gas boxes are used to create anaerobic conditions. They are

sealed chambers from which atmospheric air is pumped out by means of a vacuum pump and then filled with oxygen-free gas mixtures. Optimal for the development of obligate anaerobes is a mixture consisting of 80 % nitrogen, 10 % carbon dioxide, 10 % hydrogen. Palladium catalysts, pyrogallol or other oxygen-reducing chemicals are placed in anaerobic jar to neutralize residual oxygen.

Algorithm: "*Peculiarities of sampling the studied material in odontogenic inflammation*".

Examination of punctates.

Pathological material in abscesses, phlegmons and fasciitis is taken by puncture with a syringe, from which air is previously removed, and delivered to the laboratory by inserting a needle into a sterile rubber stopper or placing the test material in a transport medium (thioglycol, Stewart's medium).

When examining pieces of tissue, they are taken from the depths of the focus. It is also possible to take material from the depths of the focus with a standard cotton swab (for surgery). The test material is immediately immersed in the transport medium. Transport media, due to the peculiarities of their composition, provide a sharp decrease in microbial metabolism and the possibility of long-term preservation of their vital functions (from 6 to 12 hours).

DISEASES OF THE ORAL MUCOSA

Classification of stomatitis

Stomatitis is a disease of the oral mucosa of various origins, characterized by inflammation.

Infections affecting the oral mucosa and the red border of the lips can be divided into two groups: primary and secondary. The primary diseases are those in which the entrance gate of infection is the mucous membrane of the mouth and the red border of the lips, where the infectious process develops. In secondary infections, the mucous membrane is a manifestation of general, systemic human diseases such as intestinal, respiratory and others.

Infectious diseases of the oral mucosa depending on the infectious agent can be divided into bacterial, viral and fungal.

In addition, stomatitis can be divided into exogenous (infectious) and endogenous (opportunistic).

Acute bacterial infections

Purulent-inflammatory processes (boils, gingivostomatitis, scabies, chronic cracked lips, chronic ulcerative pyogenic granuloma) can be caused by various staphylococci (often *S. aureus*) and streptococci (*S. pyogenes*) (*Fig. 15*). In all of the above forms of purulent-inflammatory processes erosions with purulent discharge appear. Microtraumas can serve as an entrance gate for purulent cocci. Mixed staphylococcal and streptococcal flora is the cause of impetigo. The pustular process develops on the skin of the face, the red border of the lips and can then spread to the mucous membrane of the mouth.

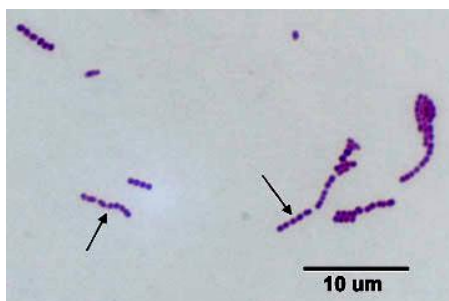


Fig. 15. Cells of *S. pyogenes*. Gram stain.

A bacteriological method is used to diagnose coccal pyodermas of the oral mucosa.

Scarlet fever. The causative agent of scarlet fever is *Streptococcus pyogenes*. The main factor of pathogenicity is erythrogenic toxin. The infection is transmitted by airborne droplets. In the patient with scarlet fever bright hyperemia of a mucous membrane of tonsils and a palate ("burning pharynx") is observed. The tongue is covered with a white plaque and mushroom-shaped papillae of bright red color stand out on this background. In severe cases, there may be ulcers. On day 2–3 of the disease, a bright pink or red small-spotted rash appears on the skin. After 10 days, changes in the oral cavity disappear. The disease is more common in preschool children.

Bacteriological method is used for diagnosis.

Gonococcal stomatitis is caused by gonococci (*Neisseria gonorrhoeae*) (Fig. 16). Unstable in the environment. Gonococcal stomatitis is transmitted by sexual and household contact ways, as well as during the passage of the child through the birth canal of an infected mother. The disease is manifested by redness, swelling of the oral mucosa, small erosions with viscous mucopurulent secretions. Ulcers, swollen and inflamed gums may be on the lips with gonorrhea. The tongue, the mucous membrane of the cheeks can be hyperemic and ulcerated. Damage to the salivary glands and pharynx is also possible.

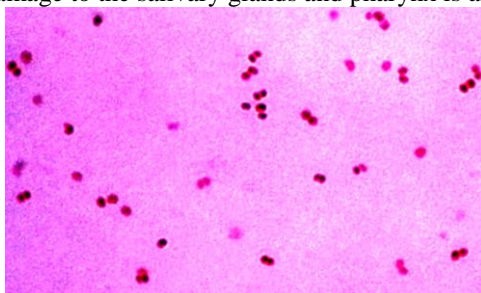


Fig. 16. Cells of *N. gonorrhoeae*. Gram stain

Bacterioscopic, bacteriological and molecular biological (PCR) methods are used for diagnosis.

Simanovsky-Plaut-Vincent's gingivostomatitis (fusospirochetosis) is referred to as opportunistic infections of the oral cavity. It is a mixed infection caused by two inhabitants of the oral cavity such as *Treponema vincentii* and *Fusobacterium nucleatum*.

The disease occurs when the protective defense of the human body is weakened (hypothermia, various stress states, hypovitaminosis, lack of secretion of sIgA on the oral mucosa). The disease is more common in young people, as well as in exhausted people on the background of smoking, chronic alcoholism and chronic diseases. But it can also develop as a complication of gingivitis or caries.

It is believed that fusospirochetosis occurs against the background of the primary inflammatory process caused by the banal coccal flora. Then there is an active reproduction of fusiform bacteria and spirochetes, which are constantly present in small quantities in the folds of the mucous membrane and gingival pockets of the oral cavity. The pathogenetic significance of fusobacteria is associated with the presence of the enzyme collagenase, which is involved in the destruction of collagen fibers of connective tissue. In this case, nitrogen-containing low molecular weight products formed as a result of collagen breakdown can be assimilated by spirochetes. Anaerobic conditions created in necrotized tissues prevent rapid recovery and contribute to further tissue damage by anaerobes (bacteroids, peptococci and peptostreptococci). The clinical picture is characterized by the formation of membranous-ulcerative lesions on the tonsils, mucous membranes of the cheeks, gums, pharynx. Diagnosis is made by bacterioscopic examination.

Diphtheria. The causative agents are toxigenic strains of *Corynebacterium diphtheriae*. Gram-positive rods with thickenings at the ends, arranged in the form of Latin letters L, Y, V. Immobile, do not form spores (*Fig. 17*). Aerobics. They remain viable in the environment for several days.



Fig. 17. Cells of *C. diphtheriae*. Methylene blue stain.

Infection occurs by airborne droplets. In the local form of diphtheria on the tonsils, palatine arches, tongue, sometimes at the site of the removed tooth and on the mucous membrane of the cheeks, white or grayish-white deposit is formed, which attaches tightly in the form of small plaques or dots. In the common form of diphtheria of the oropharynx on the background of faint redness, there are characteristic membranous plaques, the color of which becomes dirty gray or yellowish-gray. Symptoms of intoxication may be mild, and the patient suffers from the disease "on his feet." Quite often there are atypical forms of diphtheria, occurring under the guise of catarrhal, follicular or lacunar sore throat, diphtheria of the nose and wound surfaces, and the so-called "healthy" carrier of toxigenic strains often occurs.

Diagnosis of diphtheria is performed using bacteriological and bacterioscopic methods.

Chronic bacterial infections

Syphilis. The causative agent is *Treponema pallidum*. The microorganism is a thin spiral consisting of 8–12 curls. Mobile, does not form spores. Anaerobe or microaerophile. It is not stable in the environment.

It is transmitted by sexual and household contact, as well as transplacental routes.

The disease is characterized by a chronic course and stages. The formation of primary syphiloma – solid chancre – is observed after 3–4 weeks of incubation. The chancre can be located on the red border of the lips, the mucous membrane of the mouth, tongue, tonsils (chancre-amygdala). Oral chancres occur in 55 % of all extragenital sites.

The secondary period is characterized by a polymorphic rash (roseola, papules, pustules) on the skin and mucous membranes. On the mucous membrane of the oral cavity syphilitic papules are located on the cheeks along the line of closing the teeth, on the hard palate, on the tonsils in the form of dense elements with a slightly whitish smooth surface. Papules can merge into continuous erosive plaques with a grayish deposit, painless. Often similar rashes occur on the larynx, and the patient has hoarseness. Ulcers can also be found in the soft palate, tonsils and resemble aphthae, accompanied by soreness and fever.

Tertiary syphilis in the oral cavity is manifested by gumma lesions. Gumma can be localized in the mouth, on the lips and manifests itself in the form of bluish-red dense painless bumps. Syphilitic gumma in the oral cavity can be single or multiple. Gumma perforation of the hard and soft palate develops without treatment.

With congenital syphilis, the first symptoms appear as early as 1–2 months of life. The lips become swollen, thickened, yellow-red, on the surface of the affected oral mucosa ulcers appear, which later scar. Scars in the corners of the mouth (Robinson-Fournier scars) are especially characteristic.

Late congenital syphilis (after 2 years) is characterized by the Hutchinson triad: parenchymal keratitis (corneal opacity), labyrinthine deafness and Hutchinson's teeth (barrel-shaped or chisel-shaped incisors, hypoplasia of the masticatory surface with a crescent-shaped notch on the free edge). Other dystrophies of teeth are also possible: Moon's tooth (underdevelopment of chewing humps of the first molars), pike Fournier's tooth (change of a canine with thinning of its free end), etc.

Bacterioscopic and serological methods are used for microbiological diagnostics.

Tuberculosis. The causative agents of human tuberculosis are *Mycobacterium tuberculosis* and *M. bovis*. Non-motile rods, do not form a spores. Aerobics. Stable in the environment.

The main mechanism of infection are aerogenic (airborne), rarely alimentary and contact. Tuberculous lesions of the oral cavity are a manifestation of systemic disease. Most often the pathogens enter the oral cavity by hematogenous rout, and the disease manifests itself in the form of tuberculous lupus. The process is most often localized on the gums and in the area of the front teeth on the upper lip and palate. The disease begins with the appearance of a red tubercle 1–3 mm, which decays in the center with the formation of ulcers. With the further development of the disease, the bone tissue of the interalveolar septa is destroyed, which leads to mobility and tooth loss. Smooth, shiny scars are formed at the site of the lesion with a long course of the disease. Complications of the disease by bacterial or candidal secondary infection complicate the process.

Diagnosis is performed on the basis of bacterioscopic, bacteriological, biological, molecular biological and allergological methods.

Actinomycosis. Actinomycetes have the form of Gram-positive branched microorganisms with a tendency to fragmentation. Non-motile. Obligate or facultative anaerobes. *Actinomyces israelii* and *A. viscosus* are most often of pathological significance for humans. There are two points of view about the source of infection:

1. Actinomycosis is an endogenous infection that occurs when the natural resistance of a macroorganism is reduced (especially when the activity of protective factors on the mucous membranes is reduced).

2. The source of human infection can be actinomycetes, which grow on cereals, in the soil and enter the body exogenously (when injuring the mucous membranes with straw, grass, etc.).

It is now well established that potentially pathogenic actinomycetes of *A. israelii*, as a rule, live on the oral mucosa, so endogenous infection prevails over exogenous. Actinomycosis is never transmitted from person to person.

Actinomycetes are always present in the oral cavity in small quantities, but in inflammatory processes there is an increase. In the oral cavity there are favorite places of penetration of actinomycetes into the depths of tissues such

as inflamed gums near the wisdom tooth or near the destroyed roots of the teeth, pathological gingival pockets in periodontitis.

There are some clinical forms of actinomycosis, among which actinomycosis of the mandible is relatively common. Actinomycosis is characterized by the growth of granulation tissue around the microbial focus. Granulomas are formed in the soft tissues and jaw bones, which reach large sizes. The central part of the granuloma necrotizes and pus is secreted through the fistula.

Characteristic morphological granulomas called druses are found in the pus (Fig. 18). They have the appearance of yellowish grains and are the result of the development of local hypersensitivity reactions. Microscopic examination in the center of the druse shows a plexus of thin hyphae, which radially diverge in the form of rays of mycelial filaments with bulbous thickenings at the ends (contribute to the spread of actinomycetes), surrounded by eosinophilic clusters. This form (druse) has a protective value for actinomycetes, protects against phagocytosis and antibodies.

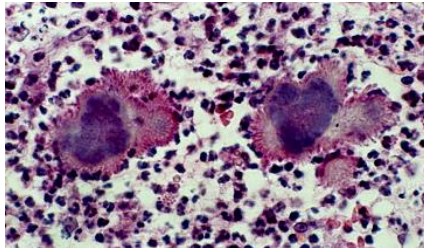


Fig. 18. Druses in actinomycosis

During the disease, as a rule, a secondary purulent infection joins, due to the penetration from the oral cavity into the lesion of various purulent microorganisms.

Diagnosis is performed on the basis of bacterioscopic and allergological methods.

Viral infections of the oral cavity

Lesions of the oral mucosa are observed in many viral infections.

Herpetic gingivostomatitis is caused by herpes simplex virus type 1 (Symplexvirus). This virus also causes herpes fever, herpes eczema, keratoconjunctivitis, meningoencephalitis and some other diseases.

Acute herpetic gingivostomatitis most often affects children from 6 months to 6 years. Primary infection with the virus occurs at this age in contact with adult carriers. The disease occurs both in the form of acute infection and activation of latent carriers.

Gingivostomatitis with herpes infection occurs with fever, severe mouth pain, salivation. At first there is a hyperemia, then vesicles are formed which quickly transform to aphthae. Clinically, it looks like an oval-shaped erosion with smooth edges, a smooth bottom covered with a grayish-whitish plaque. Aphthae are localized more often on the mucous membrane of the palate,

tongue, lips, folds. Necrotized areas of the gums are yellowish-white. Joining a coccal infection complicates the course of the disease.

Microscopic, virological, biological and serological methods are used for diagnosis.

Herpetic sore throat. The causative agent is the Coxsackie virus A (picornavirus family). The disease is manifested by vesicular rashes on the background of general redness of the oral mucosa. Vesicles burst quickly, and in their place aphthae with a grayish-white bottom are formed. The process usually ends with recovery by the end of the 1st week of the disease.

The basis of microbiological diagnostics is virological and biological methods.

Measles. The causative agent is a virus of the family Paramyxoviridae. Infection occurs by airborne droplets. Measles is characterized by the appearance whitish-yellow round spots 1–2 mm in diameter (symptom of Filatov-Koplyk) on the hyperemic mucous membrane of the cheeks near the molars, less often – on the mucous membrane of the gums or lips in the catarrhal period. With the appearance of exanthema on the skin (3–4 days) Filatov-Koplyk spots disappear. At the same time, enanthema develops on the mucous membrane of the soft palate – a rash in the form of small pale red or bright red spots that have a regular rounded or elongated shape. The presence of Koplik-Filatov spots is an absolute sign of measles.

Microscopic, virological and serological methods are used for diagnosis.

Fungal infections of the oral cavity

The causative agents of most mycoses that affect the oral mucosa are yeast-like fungi of the genus *Candida*, which cause candidiasis. The most pathogenic of all species of fungi of the genus *Candida* (there are about 150) is *C. albicans*.

Like other fungi *Candida* are eukaryotes. They can exist in the form of yeast, as well as hyphae (unseptated mycelium) and pseudohyphae (pseudomycelium) that are thin elongated cells arranged one behind the other in the form of filaments that do not have a common shell (*Fig. 19*). By type of respiration, candida is aerobic. Microorganisms are quite stable in the environment. They survive better on wet surfaces than on dry inanimate objects, but can persist for up to 24 hours with a sufficient degree of contamination.

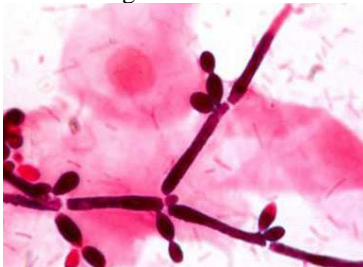


Fig. 19. Blastocystis and pseudohyphae of *C. albicans*

The most common endogenous development of candidiasis due to the carrier of fungi. Normally, a small content of these fungi is allowed in the mouth, as well as throughout the gastrointestinal tract, in the genital tract (especially in women) and on the surface of the skin. With a decrease in the resistance of mucous membranes (insufficiency of nonspecific and specific protective factors, as well as the antagonistic action of endogenous microflora), the number of yeast-like fungi begins to increase. Exogenous infection is also possible (especially in clinical hospitals). But the development of the disease occurs only against the background of immunodeficiency conditions due to age, long-term antibacterial therapy, use of corticosteroids, chemotherapy for cancer, HIV and others.

Local manifestation of candidiasis, or primary candidiasis in the oral cavity, occurs in the form of acute pseudomembranous candidiasis (thrush). The oral cavity is covered with a white plaque, which has the appearance of coagulated milk. The disease is common in newborns, especially premature and with birth injuries, as well as children who are breastfed. In adults, pseudomembranous candidiasis is rare and mainly affects people with severe secondary immunodeficiency – in cancer, after the use of steroid hormones, cytostatics, on the background of radiation therapy.

Acute atrophic candidiasis can develop as a consequence of acute pseudomembranous candidiasis. It is characterized by pain, heartburn, dry mouth. The mucous membrane of the oral cavity is fiery red, dry. When localized on the tongue, its back becomes crimson-red, dry, shiny, filamentous papillae atrophied. The plaque is absent or stored in deep folds, difficult to remove. It is a conglomerate of epithelium and a large number of fungi of the genus *Candida* in the stage of active budding (mycelium, pseudomycelium).

Chronic atrophic candidiasis often develops as a result of wearing dentures. Isolated areas of the lips (candidal cheilitis), corners of the mouth (burrs), tongue (glossitis) are mainly affected.

Hyperplastic candidiasis is characterized by the appearance on the hyperemic mucous membrane of large white papules, which sometimes merge. The mucosa of the cheeks near the corners of the lips, the back of the tongue and the back of the palate are mainly affected. The disease becomes chronic and can be considered as a precancerous disease.

Diagnosis is performed using microscopic, mycological, serological and allergological tests.

Laboratory diagnosis of stomatitis

In stomatitis of the oral cavity a plaque from a mucous membrane and tongue; material (pus, exudate) from erosions, ulcers and other elements of the lesion, as well as blood are taken as the investigated material.

The main methods of diagnosis are microscopic (bacterioscopic, viroscopic) and bacteriological (virological). In addition, serological, biological, molecular genetic and allergological methods are used.

Scraping of the mucous membrane, the back of the tongue can be done with a sterile spatula, trowel. Before taking the material from erosions, ulcers, it is necessary to remove the superficial plaque with a dry or saline swab, without using antiseptic drugs. This material can be used for microscopic and bacteriological methods of research.

In some cases, you can make smears-imprints from the mucous membrane or elements of the lesion. To do this, dry degreased glass with ground edges is applied several times to the test area. If there are hard-to-reach places, then you can use sterile columns made of rubber, which are applied first to the affected area, and then to the glass.

Collection of material from the mucous membranes and the surface of the tongue for the bacteriological method is performed with a sterile cotton swab from an area of 1 cm² and subsequent seeding on nutrient media.

General rules for sampling and transportation of samples for bacteriological examination

When taking and transporting material for microbiological research, it is necessary to follow a number of general rules:

1. Take the material before antibacterial therapy or 10–12 hours after drug withdrawal.

2. To take material directly from the center of an infection or to investigate the corresponding secretion (pus from a fistula, a sputum in pneumonia, smears from tonsils at a sore throat, etc.)

3. Take the material during the highest content of pathogens in it.

4. Follow the strictest asepsis to avoid contamination of the sample with environmental microflora.

5. Material for the isolation of aerobes and facultative anaerobes is taken by sterile cotton swabs (discharge from the wound, smears from the mucous membranes, throat), syringe (blood, pus, exudate), directly in sterile containers.

Material for the isolation of strict anaerobes is obtained from the pathological focus by puncture with a syringe, from which the air is previously removed; when examining pieces of tissue, they are taken from the depths of the hearth and immediately immersed in a transport nutrient medium. If tampons need to be used, they are also immersed in a transport medium immediately after taking the material.

6. The amount of material should be sufficient for the study and for its repetition if necessary.

7. Transportation of the native clinical sample to the laboratory should be carried out as soon as possible (no later than 2 hours after sampling), because it determines the effectiveness of microbiological research.

During long-term storage of the material, the most nutrient-demanding species of microbes die, less demanding and fast-growing species begin to multiply, which leads to violations of the quantitative ratio of species, and disorients the microbiologist in interpreting the results. However, if the material

cannot be transported to the laboratory in the next 2–3 hours, it should be stored in a refrigerator using preservatives or transport media (except for blood and cerebrospinal fluid samples).

8. Clinical specimens for the cultivation of strict anaerobes should be transported to the laboratory, protecting them as much as possible from exposure to oxygen. Use special vials filled with gas that do not contain oxygen. By injecting the needle through the rubber cap, the test material is introduced into the vial. The material can be transported directly in a syringe with a sterile stopper on the tip.

The material is also transported in transport nutrient media, for example, in a mixture of 10 % lysed donor blood, 10 % glycerol and 80 % isotonic sodium chloride solution.

It is inadmissible to transport test tubes, syringes and vials with the pathological material taken from the patient, directly in hands. All utensils containing material for microbiological examination are transported in specially designed boxes, cases, etc.

9. The clinical sample sent to the laboratory shall be accompanied by an accompanying document containing the basic information necessary for the microbiological examination (nature of the material, surname, name of the patient, name of the institution or department, medical history number, presumed diagnosis of the disease, date and time of taking the material, the name of the doctor who sends the material for examination).

Methods of taking the test material from the oral cavity

The immediate cause of most diseases of the oral cavity are residents (or their etiology is not clear) and therefore the subject of research in these cases is adapted to the body endogenous microflora, its impact on the body and the role of microbial associations in the development of opportunistic diseases. The peculiarities of the use of microbiological methods follow from this peculiarity of the pathological process.

In dental diseases as the test material can be studied: dental plaque, oral fluid, the contents of the gingival groove or pathological gingival pocket, material from the carious cavity, material from the root canals, granulomas, purulent discharge, punctures, scrapings, smears or mucous membranes elements of the lesion. In practical dentistry, smears-imprints from the mucous membrane, purulent discharge, punctures are more often examined.

General rules of material collection in dental diseases.

When taking material from different areas, saliva should be excluded from the sample. To do this, the test area is covered with sterile cotton swabs.

Before collecting the material, the oral cavity can be treated with bactericidal drugs, and it is necessary to find out from the patient whether he has not taken antibiotics in the last 3 weeks.

Due to the fact that most of the residents of the oral cavity are obligate anaerobes, the conditions of anaerobiosis must be observed during the collection of material and its transportation.

The following nutrient media are used to study the oral microbiocenosis: 5% blood agar to calculate the total microbial contamination, yolk-salt agar for staphylococci, sugar broth for streptococci, plant-milk medium for lactobacilli, Saburoud medium with polymixin for fungi, Wilson-Blair for anaerobes, Endo medium for enterobacteria.

The cultures are incubated in a thermostat for 24 hours, on Saburoud medium – about 5 days.

Identification of isolated strains of microorganisms is carried out on the basis of morphological, cultural and biochemical characteristics in accordance with the determinant of bacteria D. Bergy (1988).

Quantitative accounting population density of different ecological groups is performed by counting colony-forming units (CFU) in one gram of plaque, 1 ml of oral fluid, per 1 cm² of the surface of the tongue and mucous membranes of the cheeks, gums and palate.

Algorithm: "Study of the quantitative composition of different habitats of the oral cavity"

Quantitative accounting population density of different ecological groups is carried out by counting colony-forming units (CFU) when seeding on nutrient media.

Procedure:

1. Collection of material from the mucous membranes and the surface of the tongue for the bacteriological method is carried out with a sterile cotton swab from an area of 1 cm² and subsequent seeding on nutrient media. Oral fluid is collected in a sterile test tube, 1 ml is examined for seeding.

2. The test material is thoroughly rubbed with a loop in sector A. Then the loop is burned and from the "dirty" sector A perform 4 linear strokes in 1 sector, then also in 2 and 3 (Fig. 20).

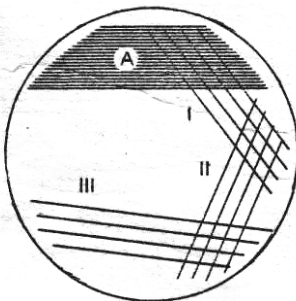


Fig. 20. Scheme of seeding of material on nutrient medium

3. The culture is incubated in a thermostat at 37 °C for 12–18 hours.

4. Count the number of colonies in the 3rd (last) sector.

The total number of viable bacteria in 1 ml of material (CFU/ml, colony-forming units) is calculated by the formula:

$N = n \cdot 10^6$, where N is the total number of bacteria, n is the number of colonies in the sector.

Terminology: autochthonous and allochthonous microflora, CFU/ml, colony-forming units, odontogenic infection, *S. mutans*, *S. salivarius*, *S. sanguis*, *S. mitis*, *S. oralis*, *S. epidermidis*, *S. aureus*, *P. niger*, *V. parvula*, *L/acidophilus*, *L. fermentum*, *L. brevis*, *L. casei*, *Neisseria*, *Bifidobacterium*, *Propionibacterium*, *Corynebacterium*, *P. buccae*, *P. denticola*, *P. melaninogenica*, *B. fragilis*, *P. oralis*, *P. oris*, *F. nucleatum*, *F. necrophorum*, *L. buccalis*, *A. israelii*, *A. naeslundii*, *A. viscosus*, *A. odontolyticus*, *B. vincentii*, *T. orale*, *T. macrodentium*, *T. denticola*, *L. dentium*, *T. pallidum*, *M. orale*, *M. salivarium*, *C. albicans*, *E. gingivalis*, *T. elongata (T. tenax)*, *T. vincentii*, *F. nucleatum*, *C. diphtheriae*, *M. tuberculosis*, *M. bovis*, *Symplexvirus*.

Practical tasks, being carried out during practical classes:

1. Gram staining and microscopy of smears of dental plaque, smear-imprint from mucosa of oral cavity and oral fluid.

2. Primary seeding of “pathogenic material” on blood agar.

3. Determination of cultural and enzymatic properties of *S. aureus*.

4. Microscopic examination of microslides of residents of the oral cavity and drawing in protocol.

5. Quantitative accounting of population density of bacteria by counting colony-forming units (CFU) when seeding on nutrient media.

6. Examination of character of growth of representatives of normal flora of oral cavity on nutrient media.

7. Studying the scheme of laboratory diagnosis of bacterial, viral and fungal infections of oral cavity.

Theoretical questions for control

1. Normal and resident microflora of the oral cavity. The role of normal oral microflora.

2. The main representatives of stabilizing species of bacteria of the oral cavity: *S. sanguis*, *S. salivarius*, *S. mitis*, *Veillonella*, *Neisseria*, *Corynebacteria*, *Lactobacilli*.

3. Anaerobic and microaerophilic streptococci. Characteristic. Role in the development of pathological processes of the oral cavity.

4. Actinomycetes, lacto- and bifidobacteria of the oral cavity. Characteristic. Role in the development of pathological processes.

5. Bacteroids of the oral cavity. Characteristic. Role in the development of pathological processes of the oral cavity.

6. Microflora in caries. Cariogenic types of microbes: microaerophilic streptococci, actinomycetes, lactobacilli. Cariogenic bacteria antagonists.
7. Microbiological methods of studying the microflora and methods of material collection in caries and its complications.
8. Microflora in periodontal disease.
9. Periodontopathogenic microbes: prevotella, porphyromonads, actinomycetes.
10. Methods and features of material collection for the study of microflora in periodontal disease.
11. The role of normal microflora in the development of odontogenic inflammation. Features of the composition of the microflora in odontogenic and neodontogenic inflammatory processes of the maxillofacial area.
12. Examination of microflora in odontogenic diseases.
13. Bacterial stomatitis. Etiology. Pathogenesis.
14. Viral and fungal stomatitis of the oral cavity. Etiology. Pathogenesis.
15. Laboratory diagnosis of stomatitis.
16. Methods of collecting pathological material from the oral cavity for microbiological examination.

Test tasks for control

1. Microscopic examination of a smear from the lesion taken from a patient with acute purulent periostitis revealed Gram-positive cocci, which are located in the form of clusters resembling bunches of grapes. Which of the following microorganisms are characterized by such morphology?

<i>A. Staphylococci</i>	<i>D. Tetracocci</i>
<i>B. Sarcina</i>	<i>E. Streptococci</i>

C. Fungy of genus Candida
2. During the examination of the patient, the dentist found white spots on the teeth – areas of enamel demineralization. Which of the following microorganisms are involved in the development of this process?

<i>A. Streptococcus mutans</i>	<i>D. Veilonella parvula</i>
<i>B. Streptococcus salivarius</i>	<i>E. Staphylococcus epidermidis</i>

C. Streptococcus pyogenes
3. Microscopic examination of dental plaque revealed cocci arranged in pairs and short chains, as well as Gram-positive rods, which may have been related to the development of caries. Which of the following associations of microorganisms are involved in the pathogenesis of caries?

<i>A. S. mutans and lactobacilli</i>	<i>D. S. mutans and corynebacteria</i>
<i>B. S. salivarius and enterococci</i>	<i>E. S. aureus and lactobacilli</i>

C. S. salivarius and lactobacilli
4. The newborn had redness, swelling of the oral mucosa and small erosions with mucopurulent discharge. Microscopic examination of smears from secretions revealed a large number of leukocytes with Gram-negative diplococci inside, as

well as the same microorganisms outside the leukocytes. Which of the following diagnoses is most likely?

- A. *Gonococcal stomatitis*
- B. *Blenorrhoea*
- C. *Staphylococcal stomatitis*
- D. *Congenital syphilis*
- E. *Toxoplasmosis*

5. Microscopic examination of the scraping of the gums in a 70-year-old patient with severe periodontitis revealed Protozoa with size of 3-60 μm with a single nucleus and broad pseudopodia. Which of the following protozoa were found?

- A. *Trichomonas tenax*
- B. *Entamoeba gingivalis*
- C. *Entamoeba histolytica*
- D. *Toxoplasma gondii*
- E. *Balantidium coli*

6. A 12-year-old boy has acute onset of disease: sore throat, body temperature rise up to 39,8 °C; on the second day diffuse skin rash was detected all over his skin except for nasolabial triangle. On examination of oral cavity: crimson tongue, "flaming pharynx", necrotic tonsillitis. Which of the following diagnosis is the most likely?

- A. *Influenza*
- B. *Scarlet fever*
- C. *Meningococemia*
- D. *Diphtheria*
- E. *Measles*

7. Inoculum from pharynx of a patient ill with angina was inoculated into blood-tellurite agar. It resulted in growth of grey, radially striated (in form of rosettes) colonies 4–5 mm in diameter. Gram-positive bacilli with clublike thickenings on their ends placed in form of spread wide apart fingers are visible by microscope. Which of the following microorganisms are these?

- A. *Streptobacilli*
- B. *Clostridia botulinum*
- C. *Corynebacteria diphtheriae*
- D. *Diphtheroids*
- E. *Streptococci*

8. Examination of a child revealed some whitish spots looking like coagulated milk on the mucous membrane of his cheeks and tongue. Analysis of smears revealed Gram-positive oval yeast-like cells. Which of the following causative agents are they?

- A. *Candida*
- B. *Fusobacteria*
- C. *Actinomycetes*
- D. *Corynebacteria diphtheria*
- E. *Staphylococci*

9. An 18-year old patient has enlarged lymphnodes. They are painless, thickened on palpation. In the area of oral mucous membrane there is a small sized ulcer with thickened edges and "laquer" bottom of greyish colour. Which of the following diseases is the most probable diagnosis?

- A. *Syphilis*
- B. *Candidiasis*
- C. *Scarlet fever*
- D. *Gonorrhoea*
- E. *Tuberculosis*

10. Microscopic examination of pus sample taken from mandibular fistula canal and stained by Gram stain has revealed druses with Gram-positive coloring in the center and cone-shaped structures with Gram-negative coloring. Such morphology is characteristic of the agent of:

A. *Candidiasis*

D. *Anaerobic infection*

B. *Actinomycosis*

E. *Syphilis*

C. *Fusobacteriosis*

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Навчальне видання

НОРМАЛЬНА МІКРОФЛОРА ПОРОЖНИНИ РОТА ТА МІКРОФЛОРА ПРИ ПАТОЛОГІЧНИХ ПРОЦЕСАХ

***Методичні вказівки з дисципліни
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для студентів II і III курсів
медичного та стоматологічного факультетів
з англійською мовою викладання***

Упорядник Коваленко Наталія Іллівна

Відповідальний за випуск

Н. І. Коваленко



Комп'ютерний набір Н. І. Коваленко
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