МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ УКРАИНЫ
Харьковский национальный медицинский университет

MICOSIS

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

МИКОЗЫ

Методические указания по дисциплине «Микробиология, вирусология и иммунология» для студентов II и III курсов медицинского и стоматологического факультетов с английским языком преподавания

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Compilers V. V. Minukhin,
N. I. Kovalenko

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

Learning guide includes sections of taxonomy, morphology and ultrastructure of fungi. The most modern information on pathogenesis, epidemiology, methods of laboratory diagnosis and prophylaxis is represented.

Микозы : метод. указ. по дисциплине «Микробиология, вирусология и иммунология» для студентов II и III курсов мед. и стомат. фак-тов с англ. языком преподавания / сост. В. В. Минухин, Н. И. Коваленко. – Харьков : ХНМУ, 2015. – 24 с.

Составители В. В. Минухин,
Н. И. Коваленко

Theme: Microbiological diagnosis of mycosis.

Actuality of the theme.

Purpose: Studying of laboratory diagnosis of mycosis.

Concrete goals:

1. Study of biological properties and classification of fungi.
2. Study pathogenesis and clinical manifestations of diseases that are caused by opportunistic fungi.
3. Study of the methods of laboratory diagnosis of diseases that are caused by opportunistic fungi.
4. Study of antifungal agents.

Students should be able to:

1. Differentiate of pathogenic fungi on morphology.
2. Isolate pure cultures of Candida albicans and examine colonies on nutrient media.
3. Identify of isolated pure culture of C. albicans on morphology, culture and biochemical properties.

Equipment: slides, immersion microscope, tables, atlas.

Fungi are ubiquitous in nature and exist as free-living saprobes that derive no obvious benefits from parasitizing humans or animals. Since they are widespread in nature and are often cultured from diseased body surfaces, it may be difficult to assess whether a fungus found during disease is a pathogen or a transient environmental contaminant. In general, fungal infections and the diseases they cause are accidental. A few fungi have developed a commensal relationship with humans and are part of the indigenous microbial flora (e.g., various species of *Candida*, especially *Candida albicans).*

Fungal infections may be classified according to route of acquisition, the site of infection, and type of virulence.

Classification Based on Route of Acquisition. Infecting fungi may be either exogenous or endogenous. Routes of entry for exogenous fungi include airborne, cutaneous or percutaneous. Endogenous infection involves colonization by a member of the normal flora or reactivation of a previous infection.

Classification Based on Site. Mycoses are classified as superficial, cutaneous, subcutaneous, or systemic (deep) infections depending on the type and degree of tissue involvement and the host response to the pathogen. Superficial mycoses are limited to the stratum corneum and essentially elicit no inflammation. Cutaneous infections involve the integument and its appendages, including hair and nails. Infection may involve the stratum corneum or deeper layers of the epidermis. Inflammation of the skin is elicited by the organism or

its products. Subcutaneous mycoses include a range of different infections characterized by infection of the subcutaneous tissues usually at the point of traumatic inoculation. An inflammatory response develops in the subcutaneous tissue frequently with extension into the epidermis. Deep mycoses involve the lungs, abdominal viscera, bones and or central nervous system. The most common portals of entry are the respiratory tract, gastrointestinal tract, and blood vessels.

Superficial and Cutaneous Mycoses

Superficial Mycoses include the following fungal infections and their etiological agent: black piedra (Piedraia hortae), white piedra (Trichosporon beigelii), pityriasis versicolor (Malassezia furfur), and tinea nigra (Phaeoannellomyces werneckii). Pityriasis versicolor is a common superficial mycosis, which is characterized by hypopigmentation or hyperpigmentation of skin of the neck, shoulders, chest, and back. Pityriasis versicolor is due to Malassezia furfur which involves only the superficial keratin layer. Black piedra is a superficial mycosis due to Piedraia hortae which is manifested by a small firm black nodule involving the hair shaft. By comparison, white piedra due to T. beigelii is characterized by a soft, friable, beige nodule of the distal ends of hair shafts. Tinea nigra most typically presents as a brown to black silver nitrate-like stain on the palm of the hand or sole of the foot.

Cutaneous Mycoses are classified as dermatophytoses or dermatomycoses. *Dermatophytoses* are caused by the agents of the genera Epidermophyton, Microsporum, and Trichophyton. *Dermatomycoses* are cutaneous infections due to other fungi, the most common of which are Candida spp. The dermatophytoses are characterized by an anatomic site-specificity according to genera. For example, Epidermophyton floccosum infects only skin and nails, but does not infect hair shafts and follicles. Whereas, Microsporum spp. infects hair and skin, but do not involve nails. Trichophyton spp. may infect hair, skin, and nails.

These fungi possess greater invasive properties than those causing superficial infections, but they are limited to the keratinized tissues. They cause a wide spectrum of diseases that range from a mild scaling disorder to one that is generalized and highly inflammatory. Studies have shown that the disease-producing potential of these agents depends on various parasite and host factors, such as the species of organism, immunologic status of the host, type of clothing worn, and type of footwear used. Trauma plays an important role in infection. These organisms gain entry and establish themselves in the cornified layers of traumatized or macerated skin and its integument and multiply by producing keratinase to metabolize the insoluble, tough fibrous protein. The reason why these agents spread no deeper is not known, but it has been speculated that factors such as cell-mediated immunity and the presence of transferrin in serum inhibit fungal propagation to the deeper tissue layers and systemic disease does not occur. Some dermatophytes have evolved a commensal relationship with the host and are isolated from skin in the absence of disease.

Subcutaneous Mycoses

There are three general types of subcutaneous mycoses: chromoblastomycosis, mycetoma, and sporotrichosis. All appear to be caused by traumatic inoculation of the etiological fungi into the subcutaneous tissue. Chromoblastomycosis is a subcutaneous mycosis characterized by verrucoid lesions of the skin (usually of the lower extremities); histological examination reveals muriform cells (with perpendicular septations) or so-called "copper pennies" that are characteristic of this infection. Chromoblastomycosis is generally limited to the subcutaneous tissue with no involvement of bone, tendon, or muscle. By comparison, mycetoma is a suppurative and granulomatous subcutaneous mycosis, which is destructive of contiguous bone, tendon, and skeletal muscle. Mycetoma is characterized by the presence of draining sinus tracts from which small but grossly visible pigmented grains or granules are extruded. These grains are microcolonies of fungi causing the infection.

The fungi that have been implicated in the subcutaneous mycoses are abundant in the environment and have a low degree of infectivity. These organisms gain access to the subcutaneous tissues through traumatic implantation. Histopathologic evidence indicates that these organisms survive in the subcutaneous tissue layers by producing proteolytic enzymes and maintaining a facultative microaerophilic existence because of the lowered redox potential of the damaged tissue. In eumycotic mycetoma there is extensive tissue damage and production of purulent fluid, which exudes through numerous intercommunicating sinus tracts. Microabscesses are common in chromoblastomycosis, but the clinical manifestation of disease indicates a vigorous host response to the organism, as seen by the intense tissue reaction that characterizes the disease (pseudoepitheliomatous hyperplasia).

Chromoblastomycosis and mycetoma are caused by only certain fungi. The most common causes of chromoblastomycosis are Fonsecaea pedrosoi, Fonsecaea compacta, Cladosporium carionii, and Phialophora verrucosa. The causes of mycetoma are more diverse but can be classified as eumycotic and actinomycotic mycetoma. Many of the fungi causing mycetoma are pigmented brown to black. These organisms are known as dematiaceous (melanized) fungi. The melanin pigment is deposited in the cell walls of these organisms. These fungi may produce a range of infections from superficial to subcutaneous to deep (visceral) infection characterized by the presence of dematiaceous hyphal and/or yeast-like cells in tissue. Such deep infections due to dematiaceous fungi are termed phaeohyphomycosis.

Sporotrichosis is the third general class of subcutaneous mycoses. This infection is due to Sporothrix schenckii and involves the subcutaneous tissue at the point of traumatic inoculation. The infection usually spreads along cutaneous lymphatic channels of the extremity involved.

The clinical manifestations of disease caused by S. schenckii vary, depending on the immune status of the patient. The classic condition,

subcutaneous lymphanigitic sporotrichosis, is characterized by numerous nodules, abscesses, and ulcerative lesions that develop along the lymphatics that drain the primary site of inoculation. The disease does not extend beyond the regional lymph nodes that drain the site of the original infection. Alternatively, infection may result in solitary lesions or pulmonary disease. Clinical manifestations of pulmonary infections vary depending on the immune status of the patient. The immunocompetent individual has a high degree of innate resistance to disease, and when infection occurs the organism is often a secondary colonizer of old infarcted or healed cavities of the lungs. If the patient is immunocompromised, dissemination can occur.

Deep (Systemic) Mycoses

Deep mycoses are caused by primary pathogenic and opportunistic fungal pathogens. The primary pathogenic fungi are able to establish infection in a normal host; whereas, opportunistic pathogens require a compromised host in order to establish infection (e.g., cancer, organ transplantation, surgery, and AIDS). The primary deep pathogens usually gain access to the host via the respiratory tract. Opportunistic fungi causing deep mycosis invade via the respiratory tract, alimentary tract, or intravascular devices.

The primary systemic fungal pathogens include Coccidioides immitis, Histoplasma capsulatum, Blastomyces dermatitidis, and Paracoccidioides brasiliensis. The opportunistic fungal pathogens include Cryptococcus neoformans, Candida spp., Aspergillus spp., Penicillium marneffei, the Zygomycetes, Trichosporon beigelii, and Fusarium spp.

Primary Mycoses

Most cases of primary deep mycoses are asymptomatic or clinically mild infections occurring in normal patients living or traveling in endemic areas. However, patients exposed to a high inoculum of organisms or those with altered host defenses may suffer life-threatening progression or reactivation of latent foci of infection.

The primary site of infection is the respiratory tract. Conidia and other infectious particles are inhaled and lodge on the mucous membrane of the respiratory tree or in the alveoli, where they encounter macrophages and are phagocytosed. To successfully colonize the host these organisms must be able to survive at the elevated temperature of the body and either elude phagocytosis, neutralize the hostility they encounter, or adapt in a manner that will allow them to multiply.

Coccidioidomycosis. The arthroconidia of C. immitis are inhaled and convert in the lung to spherules. Most cases of coccidioidomycosis are clinically occult or mild infections in patients who inhale infective arthroconidia. However, some patients have progressive pulmonary infection and also may suffer dissemination to the brain, bone, and other sites. Coccidioides meningitis is a life-threatening infection requiring lifelong treatment.

Histoplasmosis is a primary pulmonary infection resulting from inhalation of conidia of Histoplasma capsulatum which convert in vivo into the blastoconidial (budding yeast) form.

Dissemination to the hilar and mediastinal lymph nodes, spleen, liver, bone marrow, and brain may be life-threatening in infants and other immunocompromised patients. Histoplasmosis (like tuberculosis) is characterized by intracellular growth of the pathogen in macrophages and a granulomatous reaction in tissue. These granulomatous foci may reactivate and cause dissemination of fungi to other tissues. These patterns of primary infection and reactivation are similar to those of Mycobacterium tuberculosis. Histoplasmosis also may be associated with a chronic inflammatory process known as fibrosing mediastinitis, where scar tissue (formed in response to H.capsulatum) encroaches on vital structures in the mediastinum.

Blastomycosis, similar to histoplasmosis, is a primary pulmonary infection resulting from inhalation of conidia from the mycelial phase of Blastomyces dermatitidis which convert in vivo to the parasitic yeast phase. Blastomycosis (due to B. dermatitidis) in the blastoconidial phase also causes a primary pulmonary infection. The organism elicits a granulomatous reaction often associated with a marked fibrotic reaction. The clinical pattern of pulmonary blastomycosis is one of chronic pneumonia. Dissemination occurs most commonly to the skin, bone, and, in males, prostate.

Classification Based on Virulence. Primary pathogens can establish infections in normal hosts. Opportunistic pathogens cause disease in individuals with compromised host defense mechanisms.

Opportunistic Mycoses

Opportunistic mycoses are infections due to fungi with low inherent virulence means that these pathogens constitute an almost limitless number of fungi. However, when host defenses are compromised by immunosuppression (AIDS), malignancy, diabetes, organ and bone marrow transplantation, cytotoxic drugs, antibiotics, or devices that breach the normal host defenses (indwelling catheters, artificial heart valves), these usually harmless fungi become potent pathogenic microorganisms.

Candidiasis, cryptococcosis, zygomycosis, and aspergillosis are among the most common opportunistic fungal infections. These fungi are commonly observed in the environment and are innocuous to people with intact host defenses. Pneumocystis and Fusarium sp. may cause opportunistic infection too.

Candidiasis is the most common opportunistic fungal infection. C. albicansis is the most important species of Candida and causes thrush, vaginitis, skin and nail infections, and other infections. C. albicans is part of the normal flora of skin, mouth, gastrointestinal tract, and vagina. Candidiasis may be classified as superficial or deep. Superficial candidiasis may involve the epidermal and mucosal surfaces, including those of the oral cavity, pharynx,

esophagus, intestines, urinary bladder, and vagina. The alimentary tract and intravascular catheters are the major portals of entry for deep (or visceral) candidiasis. The kidneys, liver, spleen, brain, eyes, heart, and other tissues are the major organ sites involved in deep or visceral candidiasis. The principal risk factors predisposing to deeply invasive candidiasis are protracted courses of broad spectrum antibiotics, cytotoxic chemotherapy, corticosteroids, and vascular catheters.

Aspergillosis. Aspergilli are ubiquitous in the environment. They are the most frequent cause of fungal infection and death among opportunistic mycosis. Aspergilli can invade wounds, burns, abraded skin, cornea, and outer ear. The main portal of entry for aspergillosis is the respiratory tract, however, injuries to the skin may also introduce the organism into susceptible hosts. Invasive aspergillosis most frequently involves the lungs and paranasal sinuses. This fungus may disseminate from the lungs to involve the brain, kidneys, liver, heart, and bones. Blood vessel invasion can result in thrombosis and infarction. Infection of the bronchi can result in allergic bronchopulmonary aspergillosis, characterized by asthmatic symptoms. Quantitative and functional defects in circulating neutrophils are key risk factors for development of invasive aspergillosis.

Zygomycosis due to Rhizopus, Absidia, Mucor species, or other members of the class of Zygomycetes, also causes invasive sinopulmonary infections. An especially life-threatening form of zygomycosis (also known as mucormycosis), is known as the rhinocerebral syndrome. Diabetic ketoacidosis, neutropenia and corticosteroids are the major risk factors for zygomycosis. Aspergillus spp and the Zygomycetes have a strong propensity for invading blood vessels. Rhizopus infection may start in the nasal tissue but spreads rapidly to the eyes and brain.

Cryptococcosis is most typically an opportunistic fungal infection that most frequently causes pneumonia and/or meningitis. Cryptococcus neoformans occurs widely in nature, particularly in soil contaminated with bird droppings. The infection starts with inhalation of the organism and can remain localised in the lungs but often disseminates throughout the body and the most severe form is probably the non-contagious cryptococcocal meningitis when the fungus reaches the brain. Defective cellular immunity, especially that associated with AIDS, is the most common risk factor for developing cryptococcosis.

Pneumocystosis is the overall name given to infections caused by Pneumocystis, which have four clinical expressions: asymptomatic infections, infantile pneumonia (and may be associated with sudden infant death syndrome, SIDS), pneumonia in immunocompromised patients, and extrapulmonary infections. Pneumonia due to Pneumocystis is one of the most important pneumonias in immunocompromised individuals. Pneumocystis was classified as a protozoan until the late 1980s but is now widely accepted as a yeast-like fungus and molecular phylogenies place it in the Taphrinomycotina in the Ascomycota. The

original specific name given to the organism was Pneumocystis carinii but isolates causing infections in humans are now referred to P. jirovecii in honour of the Czech parasitologist Otto Jirovec. Pneumocystis carinii is still the correct name for this organism when found in hosts other than humans.

Pneumocystis DNA can be detected in air and water, although the organism may not be visible microscopically, suggesting that Pneumocystis may not survive in the environment longer than it takes to infect a susceptible host.

Pneumocystis is one of the major causes of opportunistic mycoses in immunocompromised patients, including those with congenital immunodeficiencies or AIDS, and patients receiving corticosteroid or intensive immunosuppressive therapy for treatment of cancer or prevention of transplant rejection. It is still one of the most significant AIDS-related diseases; in particular, extrapulmonary infections, resulting from dissemination of the infection from lungs to lymph nodes, spleen, bone marrow, liver, kidneys, heart, brain, pancreas, skin and other organs, occur in patients with AIDS.

Fusariosis in immunocompromised patients has also shown a rising trend. The most virulent Fusarium species is Fusarium solani (filamentous, Ascomycota). Fusarium is a well-known plant pathogen and is widely distributed on plants, including crop plants, and in the soil. The rate of infection in haematopoietic stem cell transplantation recipients has increased over time.

Structure: Fungi are eukaryotic microorganisms. They have a diploid number of chromosomes and a nuclear membrane. They possess chitinous cell walls, plasma membranes containing ergosterol, 80S rRNA, and microtubules composed of tubulin (*Fig. 1*).

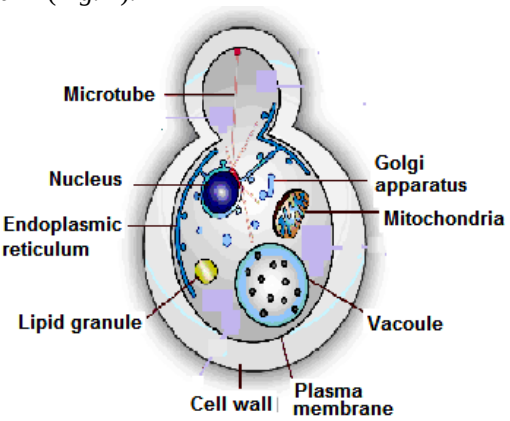

Figure 1. Structure of a cell of fungi.

The rigid cell wall of fungi is a stratified structure consisting of chitinous microfibrils embedded in a matrix of small polysaccharides, proteins, lipids, inorganic salts, and pigments that provides skeletal support and shape to the enclosed protoplast. Many fungi, especially the yeasts, have soluble peptidomannans as a component of their outer cell wall in a matrix of α- and β-glucans. Mannans are responsible for the immunologic response to the medically important yeasts and molds.

Cryptococcus neoformans produces a capsular polysaccharide.

Fungal plasma membranes are similar to mammalian plasma membranes, differing in having the nonpolar sterol ergosterol, rather than cholesterol, as the principal sterol. The plasma membrane regulates the passage of materials into and out of the cell by being selectively permeable. Membrane sterols provide structure, modulation of membrane fluidity, and possibly control of some physiologic events. The plasma membrane contains primarily lipids and protein, along with small quantities of carbohydrates.

Fungi possess microtubules composed of the protein tubulin. Microtubules are long, hollow cylinders approximately 25 nm in diameter that occur in the cytoplasm as a component of larger structures. These structures are involved in the movement of organelles, chromosomes, nuclei, and Golgi vesicles containing cell wall precursors.

The fungal nucleus is bounded by a double nuclear envelope and contains chromatin and a nucleolus. Within the cell, 80 to 99 % of the genetic material occurs in chromosomes as chromatin, and approximately 1 to 20 % in the mitochondria.

Morphology: Fungi can occur as yeasts, molds, or as a combination of both forms. Yeasts are microscopic fungi consisting of solitary cells that reproduce asexually by blastoconidia formation (budding) or fission (*Fig. 2*). Molds reproduce asexually and/or sexually. They form multicellular hyphae that elongate by a process known as apical elongation, which requires a careful balance between cell wall lysis and new cell wall synthesis. Hyphae can be sparsely septate to regularly septate and possess a variable number of nuclei. Some molds produce special sac-like cells called sporangia, the entire protoplasm of which becomes cleaved into spores called sporangiospores (*Fig. 3*). Sporangia are typically formed on special hyphae called sporangiophores.

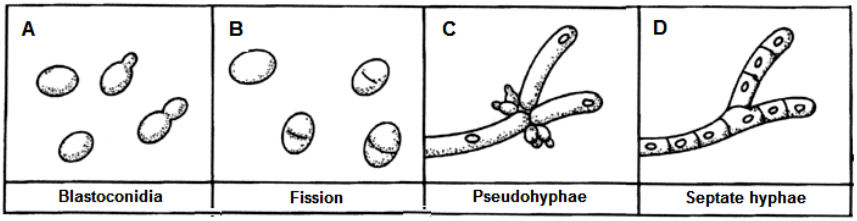

Figure 2. A. Yeast cells reproducing by blastoconidia formation; B. Yeast dividing by fission; C. Pseudohyphal development; D. Septate hyphae

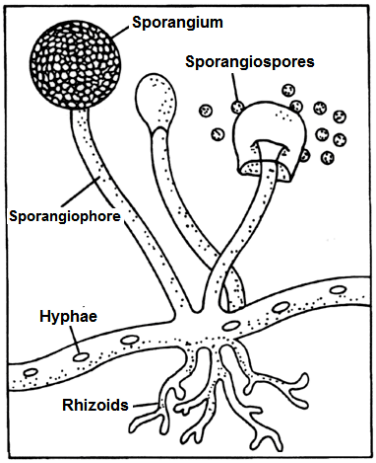

Figure 3. Asexual fruiting structure of Rhizopus species, illustrating sporangium, sporangiophore, sporangiospores, coenocytic hyphae and rhizoids

Yeasts such as C. albicans and C. neoformans produce budded cells known as blastoconidia (*Fig. 2*). Formation of blastoconidia involves three basic steps: bud emergence, bud growth, and conidium separation. During bud emergence, the outer cell wall of the parent cell thins. Concurrently, new inner cell wall material and plasma membrane are synthesized at the site where new growth is occurring. New cell wall material is formed locally by activation of the polysaccharide synthetase zymogen. The process of bud emergence is regulated by the synthesis of these cellular components as well as by the turgor pressure in the parent cell. Mitosis occurs, as the bud grows, and both the developing conidium and the parent cell will contain a single nucleus. A ring of chitin forms between the developing blastoconidium and its parent yeast cell. This ring grows in to form a septum. Separation of the two cells leaves a bud scar on the parent cell wall. The bud scar contains much more chitin than does the rest of the parent cell wall. When the production of blastoconidia continues without separation of the conidia from each other, a pseudohypha, consisting of a filament of attached blastoconidia, is formed. In addition to budding yeast cells and pseudohyphae, yeasts such as C. albicans may form true hyphae.

A mass of hyphal elements is termed the mycelium (synonymous with mold). Aerial hyphae often produce asexual reproduction propagules termed conidia (synonymous with spores) (*Fig. 4*). Relatively large and complex conidia are termed macroconidia while the smaller and more simple conidia are termed microconidia. When the conidia are enclosed in a sac (the sporangium), they are called endospores. The presence/absence of conidia and their size,

shape and location are major features used in the laboratory to identify the species of fungus in clinical specimens (*Fig. 5*).

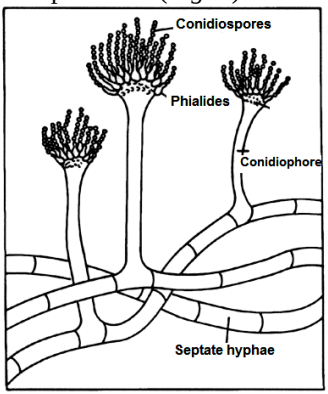

Figure 4. Asexual fruiting structure of Aspergillus species, illustrating septate hyphae, conidiophore, vesicle, phialides and conidiospores

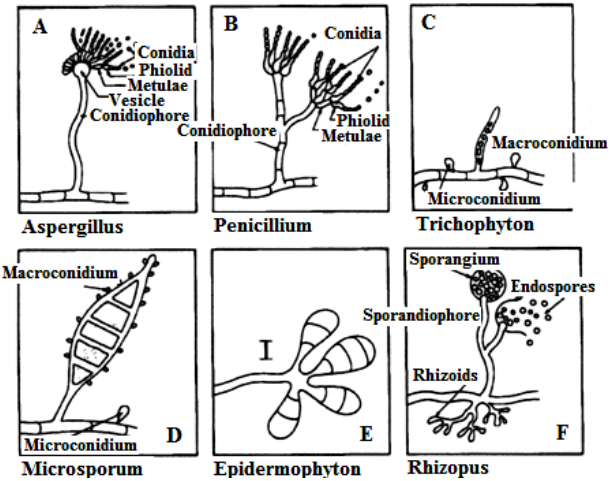

Figure 5. Differentiation of species of fungus in clinical specimens: A. Aspergillus; B. Penicillium; C. Trichophyton; D. Microsporum; E. Epidermophyton and F. Rhizopus

Asexual reproduction, via conidia formation, does not involve genetic recombination between two sexual types whereas sexual reproduction does involve genetic recombination between two sexual types.

Some of the opportunistic fungal pathogens of humans are dimorphic, growing as a mycelium in nature and as vegetatively reproducing yeast in the

body. Candida is an example of such a dimorphic fungus. It can undergo rapid transformation from the yeast to the hyphal phase in vivo, which partly contributes to its success in invading host tissue. C. albicans may form a budding yeast, pseudohyphae, germ tubes, true hyphae, and chlamydospores. C.albicans has three serotypes, designated A, B, and C. These are distinguished from each other on the basis of their mannans.

Dimorphism in the Pathogenic Fungi

Fungal dimorphism is the morphological and physiological conversion of certain fungi from one phenotype to another when such fungi change from one environment to another. Dimorphic means two forms; pleiomorphic means more than one form. The terms are applied to fungi because they can have different forms, of hyphae or spores. Most fungal pathogens do take yeast forms once they are invaded human tissues.

At the most obvious, fungi commonly have sexual and asexual stages in their life cycles. What is less commonly understood, is that some fungi may have many morphologically different types of asexual spore. Further, the thallus of some fungi may reversibly switch from spheres (yeast cells), to elongated conjoined buds (pseudohyphae), to filamentous (hyphae) under specific environmental conditions (temperature, energy, organic nitrogen, pH, oxygen, osmotic potential, minerals such as Ca etc). These forms are independent of one another and have quite specific developmental characteristics.

Many fungi produce two different spore types during asexual proliferation. Unfortunately, these states can cause confusion when the spore resembles those of other fungi. Similarity of form does not necessarily indicate relatedness, and care must be taken when naming fungi using asexual structures.

The nature of each form may also be under genetic control. Many fungi modify their growth according to the environmental conditions under which they grow. For instance, soil fungi have a sparse filamentous growth in culture when organic carbon is poorly available. When organic carbon is plentiful, the hyphae branch frequently, and the mycelium is dense. The net effect for the fungus is that in conditions of plentiful energy, the fungus is able to absorb large quantities, but when food is lacking, little energy is spent on exploring the location. In addition, different parts of a single mycelium may exhibit different forms: dense colonisaion of attractive and sparce colonisation (including presence of strands) of unattractive habitats. The main consequence of the variation in growth patterns is that fungi in soil thoroughly explore and efficiently exploit the highly variable and dynamic oligotrophic environment.

True dimorphic growth is found in a number of fungi that cause disease in mammals, insects and plants. Outside the body, the fungus is found as a filamentous mycelium. In general, the hyphae have the potential to invade bodies. They may also penetrate and ramify through solid substrates. However,

the filamentous form lacks a capacity to be dispersed through the organism unless it can fragment. Fragmentation requires a major switch in form.

Dimorphic fungi include C. immitis, H. capsulatum, B. dermatitidis, P. brasiliensis, P. marneffei, and S. schenckii, and certain opportunistic fungi such as Candida albicans and Penicillium marneffei. Various environmental host factors control fungal dimorphism. These factors include amino acids, temperature, carbohydrates, and trace elements (e.g. zinc). A range of pathogenic fungi of humans enter the lungs where they cause inflammation. The pathogens grow as mycelium in the tissue and eventually enter the blood stream of immunocompromised individuals where they immediately assume a yeast-like form. The fungi Histoplasma, Blastomyces and Paracoccidioides convert to yeast forms at 37°C.

Among the primary pathogens and S. schenckii, the morphological transformation is from a hyphal form to a yeast-like form (or spherule in the case of C. immitis) in tissue (*Fig. 6*). However, the dimorphism of Candida albicans is somewhat different in that the organism transforms from a budding yeast-like structures (blastoconidia) to filamentous structures known as germ tubes (*Fig. 7*). When the hyphae are constricted at the septum, they are called pseudohyphae, and when the hyphae are cylindrical, they are called true hyphae. Hyphae and pseudohyphae have one distinct difference: a chain of pseudohyphal cells divide at the same time by budding, whereas hyphae elongate from the tip, and they have a Spitzenkorper. Yeast cells and pseudohyphae are differentiated on the basis of their form: they have similar mechanisms for dividing. These forms are regulated by different genes as mutants may develop to two of three forms.

Blastomyces dermatitidis. The conversion of the mycelial form of Blastomyces dermatitidis to the large, globose, thick-walled, broadly based budding yeast form requires only increased temperature. Hyphal cells enlarge and undergo a series of changes resulting in the transformation of these cells into yeast cells. The cells enlarge, separate, and then begin to reproduce by budding.

Coccidioides immitis is a unique dimorphic fungus because it produces spherules containing endospores in tissue, and hyphae at 25°C. Increased temperature, nutrition, and increased carbon dioxide are important for the production of sporulating spherules. A uninucleate arthroconidium begins to swell and undergo mitosis to produce additional nuclei. Once mitosis stops, initiation of spherule septation occurs. The spherule is segmented into peripheral compartments with a persistent central cavity. Uninucleate endospores occurring in packets enclosed by a thin membranous layer differentiate within the compartments. As the endospores enlarge and mature, the wall of the spherule ruptures to release the endospores. Pairs of closely appressed endospores that have not completely separated from each other may resemble the budding yeast cells of B. dermatitidis.

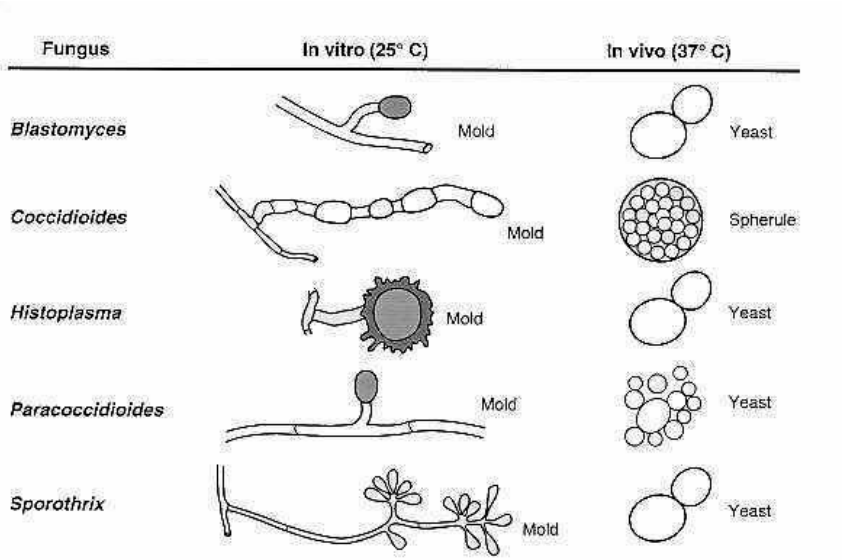

Figure 6. Diagrammatic representation of the saprophytic and invasive tissue forms of pathogenic fungi.

Histoplasma capsulatum. Dimorphism in H. capsulatum involves three stages. The conversion of terminal or intercalary hyphal cells to a yeast form requires 3 to 14 days. In tissue, H. capsulatum proliferates within giant cells.

Multiplication. Fungi may reproduce sexually or asexually. Spores can be produced either asexually or sexually. Asexual spores are always formed in a sporangium following division of nuclei by mitosis and cytoplasmic cleavage. The number of sporangiospores and their arrangement in the sporangium are used to differentiate the various zygomycetes. Sexual reproduction occurs by the fusion of two haploid nuclei (karyogamy), followed by meiotic division of the diploid nucleus. Ascospores are formed in a saclike cell (called an ascus) by free-cell formation, basidiospores form on basidia, and zygospores form within zygosporangia. Sexual spores are rarely seen in clinical isolates because most fungi are heterothallic (i.e., sexually self-sterile). Typically, only one of the two mating types is isolated from a particular clinical specimen. When homothallic isolates are recovered in the clinical laboratory, they often produce sexual spores because they are sexually self-fertile.

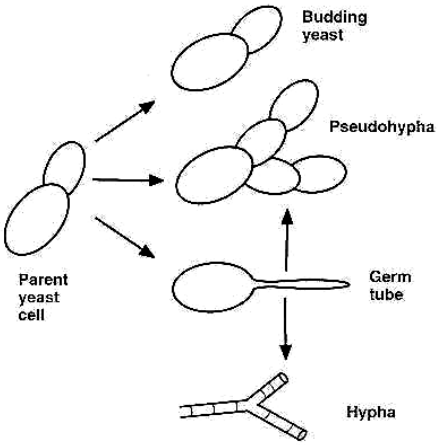

Figure 7. Germination of Candida albicans.

Conidia are always asexual in origin and develop in any manner that does not involve cytoplasmic cleavage. The ontogeny of conidia (conidiogenesis) and their arrangement, color, and septation are used to differentiate the various genera of molds. Some fungi have melanin in the cell wall of the conidia, the hyphae, or both. Such fungi are considered to be dematiaceous. Many of the name changes that have been recently proposed reflect a better understanding of conidiogenesis.

In mycology, fungi are classified on the basis of their ability to reproduce sexually, asexually, or by a combination of both. *Asexual reproductive structures,* which are referred to as *anamorphs,* are the basis for one of the sets of criteria. Because the criteria are based upon asexual morphologic forms, this system does not reflect phylogenetic relationships. It exists so that we can communicate in a simple and consistent manner by using names based upon similar morphologic structures. The second set of criteria is based upon *sexual reproductive structures*, which are referred to as *teleomorphs*. Ascospores, basidiospores, oospores, and zygospores, as well as any specialized structures associated with their development, are the basis of the second set of criteria. These criteria reflect phylogenetic relationships because they are based upon structures that form following meiosis. The term *holomorph* is used to describe the whole fungus, which consists of its teleomorph and anamorphs.

For example, the dimorphic fungus Blastomyces dermatitidis produces two anamorphs, one consisting of hyphae and one-celled conidia at 25°C and one consisting of budding yeast cells at 37°C. The name B. dermatitidis summarizes these two anamorphs. When two sexually compatible isolates of B.dermatitidis

are mated under the appropriate conditions, a sexual fruiting body, called a gymnothecium, containing ascospores will develop. The name that is used for this sexual form or teleomorph is Ajellomyces dermatitidis. When one wishes to refer to the whole fungus, the name for the teleomorph is used because it reflects phylogenetic relationships. It is important to note that the name B.dermatitidis can be used whenever one wishes to refer to the hyphal or yeast forms of this fungus.

Cell Separation (Budding). Under favorable living conditions, yeast multiplies through the separation of cells (budding) or yeast multiplication. The cell core migrates to the cell wall of the yeast cell. It splits up and forms a daughter cell. The daughter cell multiplies in the same way while it is still growing and tied to the mother cell. A colony develops. Later, the daughter cell separates from the mother cell. The multiplication process continues for as long as the conditions for multiplication are present (*Fig. 8*). As can be seen, a parent cell grows a protuberance, this swells as the bud forms, a neck develops between the parent cell and the bud, and they separate. The process starts again and, in ideal conditions, a cell can reproduce itself in 20 minutes so that numbers increase from one to two, then to four, to eight, to 16, and so on. If the numbers are plotted on a graph, the line would take an exponential form.

Figure 8. Yeast cell budding

Pneumocystis trophic (vegetative) forms are produced during asexual growth; this is called a trophozoite, the terminology surviving from the time the organism was thought to be a protozoan. Trophozoites are variable in morphology and occur clustered together in the host tissue. They are probably haploid and capable of replicating asexually by mitosis, and they produce a diploid zygote by conjugation. Meiosis occurs in the zygote, forming a precyst initially, then an early cyst and, finally, a mature cyst (again, terminology surviving from protozoan days); during this maturation process eight intracystic spores or daughter cells are produced, which must be ascospores resulting from meiosis followed by a mitosis. These spores are released when the mature cyst ruptures and germinate into trophic forms. How this cycle relates to release of an infective agent to the environment is unknown.

Physiology: Fungi are essentially aerobic, with limited anaerobic capabilities, and can synthesize lysine. They are all heterotrophic and digest

their food externally by releasing hydrolytic enzymes into their immediate surroundings (absorptive nutrition).

Fungi can use a number of different carbon sources to meet their carbon needs for the synthesis of carbohydrates, lipids, nucleic acids, and proteins. Oxidation of sugars, alcohols, proteins, lipids, and polysaccharides provides them with a source of energy. Differences in their ability to utilize different carbon sources, such as simple sugars, sugar acids, and sugar alcohols, are used, along with morphology, to differentiate the various yeasts. Fungi require a source of nitrogen for synthesis of amino acids for proteins, purines and pyrimidines for nucleic acids, glucosamine for chitin, and various vitamins. Depending on the fungus, nitrogen may be obtained in the form of nitrate, nitrite, ammonium, or organic nitrogen; no fungus can fix nitrogen. Most fungi use nitrate, which is reduced first to nitrite (with the aid of nitrate reductase) and then to ammonia.

Entry: Fungi infect the body through several portals of entry. The first exposure to fungi that most humans experience occurs during birth, when they encounter the yeast C. albicans while passing through the vaginal canal. During this process the fungus colonizes the buccal cavity and portions of the upper and lower gastrointestinal tract of the newborn, where it maintains a life-long residence as a commensal.

C. albicans is considered part of the indigenous flora. Only under certain unusual circumstances have they caused disease. It is generally thought that when C. albicans is unable to adhere to mucosa it is nonpathogenic and that production of germ tubes or hyphae plays a major role in colonization and infection of the mucosal epithelial cells by allowing direct penetration of these cells with specific hydrolytic enzymes.

Other fungi that have been implicated in human diseases come from exogenous sources, where they exist as saprobes on decaying vegetation or as plant parasites. Opportunistic fungi rarely cause disease in healthy, immunocompetent hosts. It is only when fungi accidentally penetrate barriers such as intact skin and mucous membrane linings, or when immunologic defects or other debilitating conditions exist in the host, that conditions favorable for fungal colonization and growth occur.

The yeast cells of Cryptococcus neoformans may become desiccated in the environment such that their small size allows inhalation deep into lung tissue. Germination of spores or vegetative growth of yeast cells in lung tissue results in the proliferation of budding cells and the formation of giant cells in a fraction of the population

Pathogenicity: Fungal pathogenesis is complex and involves the interplay of many factors. Although most fungal diseases are the result of accidental encounters with the agent, many fungi have developed mechanisms that facilitate their multiplication within the host.

Several factors contribute to infection and pathogenesis of primary deep pathogenic agents. Of the six systemic agents, five, Histoplasma capsulatum, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis, and Penicillium marneffei are dimorphic, changing from a mycelial to a unicellular morphology when they invade tissues, except C. immitis that forms spherules. The change from mycelial to yeast morphology in H.capsulatum appears critical for pathogenicity. Several physiologic changes occur in the fungus during the transition, which is induced by the temperature shift to 37°C. The triggering event is a heat-related insult: the temperature rise causes a partial uncoupling of oxidative phosphorylation and a consequent decline in the cellular ATP level, respiration rate, and concentrations of electron transport components. The cells enter a period of dormancy, during which spontaneous respiration is maintained at a decreased level. Then there is a shift into a recovery phase, during which transformation to yeast morphology is completed. Mycelial cells of H. capsulatum that are unable to undergo this morphologic transition are avirulent. Similar observations have been made when mycelia of B. dermatitidis and P. brasiliensis are shifted from 25°C to 37°C, and it has been implied that transformation to the yeast morphology is critical for infection.

Candida is usually described as a dimorphic fungus, although it would be better described as pleomorphic with a range of morphologies, from ovoid yeast cells at one extreme to filamentous hyphae at the other (*Fig. 9*). When C. albicans is grown in medium containing serum it forms pseudohyphal germ tubes, not buds. This is used as a diagnostic feature. The hyphal (or pseudohyphal) phase has several advantages over the yeast form because it is: more pathogenic than the yeast phase, more efficient at penetrating epithelial layers, and more resistant to defense systems.

C. immitis is also dimorphic, but its parasitic phase is a spherule. Dimorphism does not appear to play a role in C.neoformans pathogenesis since the organism is an encapsulated yeast both at 25°C and in host tissues. Acapsular variants of the yeast are either avirulent or markedly deficient in pathogenicity. Encapsulated C. neoformans cells are highly resistant to phagocytosis by human neutrophils, whereas acapsular variants are effectively phagocytosed. In addition, the capsular polysaccharide is poorly immunogenic in humans. The sexual phase of C. neoformans, Filobasidiella neoformans, is known, and the organism assumes a filamentous morphology, producing small basidiospores. It has been suggested that these propagules are relevant in infection.

The infectious propagules of H. capsulatum, B. dermatitidis, P. brasiliensis, and C. immitis are readily phagocytosed by alveolar macrophages. To survive phagocytosis and to multiply, these fungi must neutralize the effects of the phagocytes. The production of reactive oxygen metabolites by phagocytic cells is an important host defense against microorganisms. Arthroconidia of C.immitis inhibit phagosome-lysosome fusion and survive within normal

murine peritoneal macrophages. Phagosome-lysosome fusion takes place after H. capsulatum infection, but the yeast cells survive in the phagolysosome.

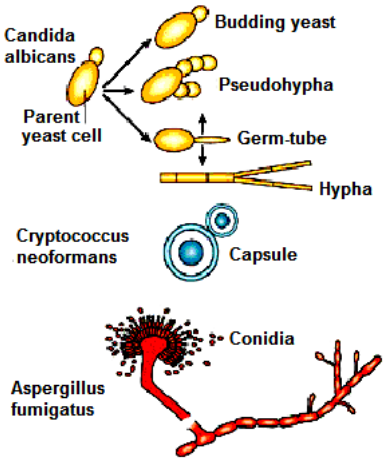

Figure 9. Diagrammatic representation of the saprophytic and invasive tissue forms of opportunistic fungi.

Host Defenses: The high degree of innate resistance of humans to fungal invasion is based primarily on the various protective mechanisms that prevent fungi from entering host tissues. Fungal growth is discouraged by the intact skin and factors such as naturally occurring long-chain unsaturated fatty acids, pH competition with the normal bacterial flora, epithelial turnover rate, and the desiccated nature of the stratum corneum. Other body surfaces, such as the respiratory tree, gastrointestinal tract, and vaginal vault, are lined with mucous membranes (epithelium) bathed in fluids that contain antimicrobial substances, and some of these membranes are lined with ciliated cells that actively remove foreign materials. Only when these protective barriers are breached can fungi gain access to, colonize, and multiply in host tissues. Fungi gain access to host tissues by traumatic implantation or inhalation. The severity of disease caused by these organisms depends upon the size of the inoculum, magnitude of tissue destruction, the ability of the fungi to multiply in tissues, and the immunologic status of the host.

Both humoral and cell mediated immunity (CMI) are important in control of fungal infections, but CMI appears to be more important since patients with defects in CMI usually suffer more severe fungal infections than do persons with depressed humoral immunity.

Laboratory diagnosis: The first suspicion of a fungal infection is often the result of the clinical presentation. Microscopic examination of fungal isolates is essential to the identification of the organism.

Microscopic examination of skin scrapings, sputum, blood, liquor, a vaginal discharge or bronchoscopic washings might reveal fungi.

C. albicans is distinctive in that it produces germ tubes and chlamydospores. It may appear in tissue as budding yeast or as elongated pseudohyphae (nonseptate) (*Fig. 10*).

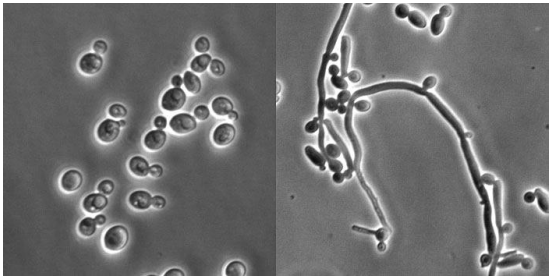

Figure 10. Microphotograph of C. albicans.

The lack of septate hyphae on a direct smear may be the initial hint of zygomycosis. Rhizopus species have sporangia that arise from a stolon. Aspergillosis, caused by a number of species of Aspergillus, is characterized in direct smear by septate hyphae, dichotomously branched. The presence of encapsulated yeast in clinical specimens may suggest the presence of Cryptococcus.

India ink preparations of CSF of patient with cryptococcal meningitis reveal a budding yeast with a wide, unstained capsule in infected persons.

Culture is frequently used in combination with direct microscopic examination of specimens and/or biopsies in order to confirm the diagnosis and to identify the responsible organism.

Special media for C. albicans:

Sabouraud solid medium: glucose or maltose, peptone, agar, pH 6,6-7,2; to inhibit contaminants penicillinum, streptomycin and chloramphenicol.

Sabouraud liquid medium: the same ingredients without agar.

Potato agar: boiled potatoes, agar, glucose.

Brain heart medium: the extract from bovine brain and heart muscle, pepton, glucose, NaCl.

Hiss media for determination of enzymatic activity.

Incubation is preferably carried out at 25-30 °C for 24-48 hours.

Colonies: When grown on solid media C. albicans forms large convex colonies of white or cream-colored creamy consistency; yeast cells are oval or oblong-oval size of 2.9-7.2 x 2.9-14.4 mm.

As the depletion of glucose in the medium chlamydospores form on the terminal filaments of pseudomycelium. Chlamydospores have granular content and their diameter exceeds the diameter of the carrier cells almost 2-fold.

When grown on liquid media with proteins (serum, plasma, egg yolk) C.albicans grows and produces germ tubes within 2-4 hours at 37°C. Some strains of C. albicans that do not produce germ tubes are avirulent.

Identification of Candida species is based on character of growth in liquid media and colonies appearance, enzymatic activity, type of filamentation.

Serology: For some fungal diseases serology is used to detect fungal antigens or host antibodies.

Agglutination test for diagnosis of candidiasis is considered true positive for serum dilution more than 1:100. CFT is less sensitive but more specific.

Indirect immunofluorescence has high diagnostic value. Diagnostic titer is 1:80. Titer of IF for healthy carriers is 1:10 – 1:20.

Control of fungal infections may include prevention as well as treatment. Prevention includes avoidance of environments and conditions conducive to fungal growth. Maintenance of a "spore-free" environment in hospitals can reduce the incidence of nosocomial fungal infections. In the case of immunocompromised patients, management of the underlying disease also may help reduce the incidence of fungal infections (e.g. control of diabetes via diet, oral hypoglycemics or insulin). In other situations such as AIDS, cancer patients receiving chemotherapy or organ transplant recipients, prophylaxis with antifungal agents may be necessary.

Whether used for prophylaxis or treatment, many antifungal drugs take advantage of the fact the sterol in the fungal cell membrane is ergosterol instead of cholesterol as in humans. Inhibitors of ergosterol biosynthesis include azoles (fluconazole, clotrimazole, itraconazole, miconazole), allylamine and morpholine antifungal drugs. The polyenes form complexes with ergosterol in the membrane.

Practical tasks, being carried out during practical classes:

1. Studying microslides: C. albicans (stained after Gram).
2. Studying colonies of C. albicans on Sabouraud agar.
3. Studying the scheme of laboratory diagnosis of candidiasis.

Therminology: yeast, mold, chlamydospore, conidia, hyphae, Sabouraud agar, Hiss media.

Theoretical questions for control:

1. Classification of fungi.
2. Genera Candida, Cryptococcus, Aspergillus, major characteristics, cellular structure.
3. Routes of transmission and pathogenesis of infections, caused by fungi.
4. Culture properties of C. albicans.
5. Laboratory diagnosis of candidiasis.

REFERENCES

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

2. Tsyganenko A. Ya. Practical exercises of morphology of bacteria. Short textbook for students of high medical schools / A. Ya. Tsyganenko, V. N. Vasilchenko, N. I. Kovalenko. – Kharkiv : KSMU, 2015. – 284 p.

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

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

ADDITIONAL REFERENCES

1. Gupte Satish. The Short Textbook of Medical Microbiology / Satish Gupte. – 9th Ed. – New Delhi: Jaypee Brothers Medical Publishers Ltd., 2006. – 509 p.

2. Manual of Clinical Microbiology / P. R. Murray et al. – 9th Ed. – Washington: ASM Press, 2007. – 2396 p.

3. Hoog G. S. De. Atlas of Clinical Fungi / G. S. De Hoog. – 2nd Ed. – Amer Society for Microbiology, Science, 2000. – 1126 p.

4. Moore D. 21st Century Guidebook to Fungi / D. Moore, G. D.Robson, A. P. J. Trinci // Cambridge, UK: Cambridge University Press, 2011. – Access: http://www.cambridge.org/gb/knowledge/isbn/item6026594/?site_locale=en_GB.

5. Nucci M. Emerging fungal diseases / M. Nucci, K. A. Marr. // Clinical Infectious Diseases. – 2005. – № 41. – P. 521-526. – Access: http://dx.doi.org/10.1086/432060.

6. CDC - Fungal Diseases. – Access: http://www.cdc.gov/fungal/diseases/index.html

7. Spectrum of Mycoses / Thomas J., Walsh Dennis, M. Dixon. Access: http://www.njmoldinspection.com/mycoses/Spectrum%20of%20Mycoses.htm

8. Ben E. de Pauw. What Are Fungal Infections? / Ben E. de Pauw // Mediterranean Journal of Hematology and Infectious Diseases. – 2011. – V. 3, № 1. – Access: http://www.mjhid.org/index.php/mjhid/rt/printerFriendly/263/310

9. Nyirjesy P. Genital mycotic infections in patients with diabetes / P. Nyirjesy, J. D. Sobel // Postgrad. Med. – 2013. – V. 125, № 3. – P. 33–46.

10. The Fungi – General Properties. In: Medical Microbiology Fall. Dr. Tritz's Lectures. – 2000. – Access: http://www.atsu.edu/faculty/chamberlain/website/lects/fungi.htm

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Составители Минухин Валерий Владимирович
Коваленко Наталья Ильинична

Ответственный за выпуск В. В. Минухин

Компьютерная верстка Е. Ю. Лавриненко
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izdatknmu@mail.ru, izdat@knmu.kharkov.ua

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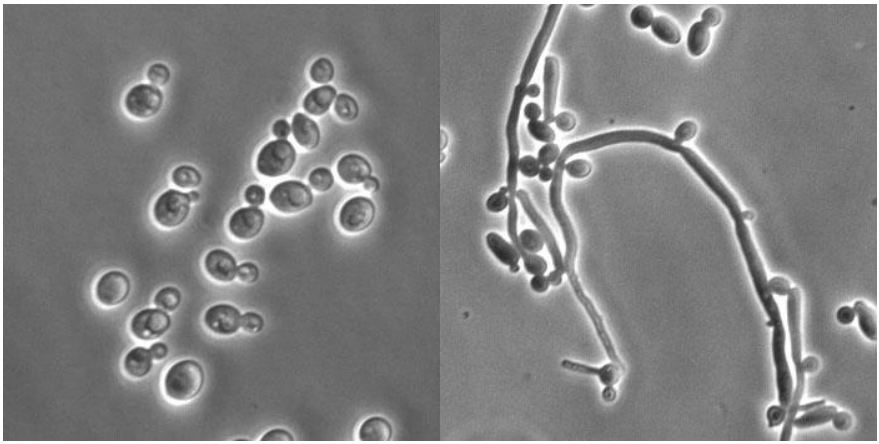

MYCOSIS

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