SUBJECT: MEDICAL MICROBIOLOGY *Aleksandar M Džamić*, April 2020
SECTION: HUMAN PARASITOLOGY & MEDICAL MYCOLOGY
PART: MEDICAL MYCOLOGY
TOPIC: MYCOLOGY EXERCISE

1. mycelium is mass or mat of hyphae, mold colony

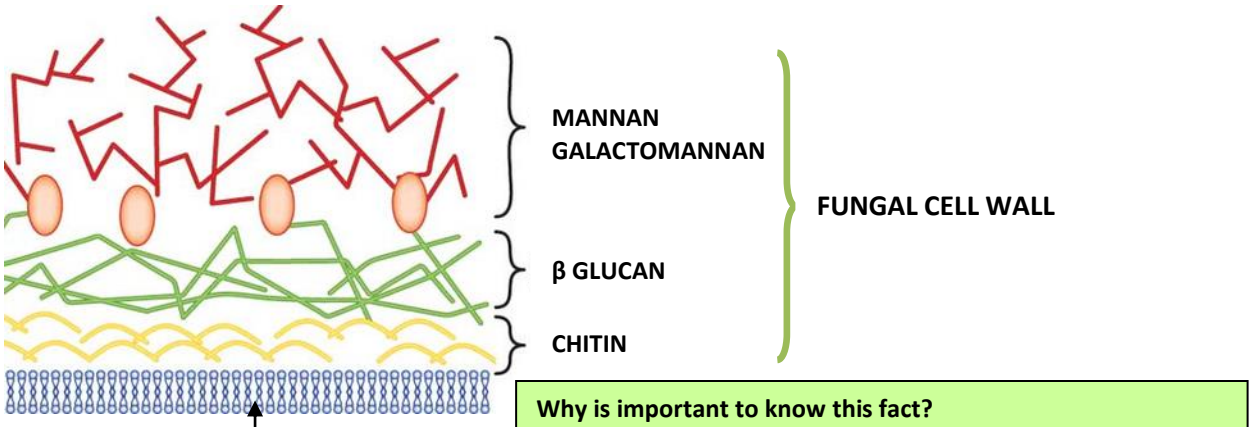
Comment:
Fungi grow in two basic forms, as **yeasts (unicellular - single cell fungi, eg *Candida*)** and **molds** (or **moulds; multicellular fungi, filamentous fungi, eg *Aspergillus*)**. Growth in the mold form occurs by the production of multicellular fiamentous colonies. These colonies consist of branching cylindric tubules called **hyphae** (filaments), varying in diameter from 2 to 10 µm. The mass of intertwined hyphae that accumulates during active growth is a **MYCELIUM**. Some hyphae are divided into cells by cross-walls or **septa**, which typically form at regular intervals during hyphal growth. However, members of the Order *Mucorales* (please, see additional notes at the end) produce hyphae that are rarely septated. **Vegetative or substrate hyphae (VEGETATIVE MYCELIUM)** penetrate the supporting medium, anchor the colony, and absorb nutrients. In contrast, **aerial hyphae (AERIAL MYCELIUM)** project above the surface of the mycelium and usually bear the reproductive structures of the mold. When a mold is isolated from a clinical specimen, its growth rate, macroscopic appearance, and microscopic morphology are usually sufficient to determine its genus and sometimes even species.
Some single cell fungi (eg. *Candida*) form structures called **pseudohyphae** which are the chains of elongated yeast cells.

2. sexual and asexual reproduction; asexual reproductive elements are termed **conidia (eg. blastoconidia in yeasts - *Candida*, eg. macro- and microconidia in molds – dermatophytes)** & sexual reproductive structures are called **spores**

3. asexual reproduction in human body

4. chitin is a component of fungal cell wall (inert, insoluble, provides structural support)

Comment:
Fungi have an essential rigid **cell wall** which is composed largely of carbohydrate layers - long chains of polysaccharides — as well as glycoproteins and lipids. Some sugar polymers are found in the cell walls of many fungi, such as **chitin** (an unbranched polymer of β-1,4-linked *N*-acetylglucosamine); **β glucans**, which are glucose polymers (eg. β-1,3-glucan and β-1,6-glucan); and **mannans**, polymers of mannose (eg. α-1,6-mannose). In addition, other polysaccharides may be unique to specific fungal species. Chitin, mannan and glucan are in close association with each other and with structural proteins.



FEATURES OF FUNGAL CELL WALL AND ITS COMPONENTS !!!

- ✓ determines fungal **shape** and **protects** from osmotic and environmental stress
- ✓ surface components of the cell wall mediate **attachment** of the fungus to host cells
- ✓ specific fungal cell wall moieties bind to pattern recognition receptors on host cell membranes, such as certain toll-like receptors, to **stimulate innate immune responses**
- ✓ cell wall glucans may activate the complement cascade and provoke an **inflammatory reaction**
- ✓ cell walls also release **immunodominant antigens** that may elicit cellular immune responses and diagnostic antibodies !!!
- ✓ some **antifungal drugs** act on the synthesis of the cell wall components (eg. glucan)

Practical use of knowledge about fungal cell wall - Diagnostic Tools – Detection of Biomarkers (Abs & Ags)
When invasive (systemic) fungal disease is suspected we can perform diagnostic tests, eg ELISA . . ., for the detection of cell wall **antigens** (mannan, galactomannan, β glucan) in patient's blood and other fluids & **antibodies** (IgM, IgG) against those antigens.
eg 1. antimannan Abs and mannan Ag if ***Candida*** infection is suspected
eg 2. antigalactomannan Abs and galactomannan Ag if ***Aspergillus*** infection is suspected
eg 3. glucan Ag as a **common Ag** if ***Candida*****,** ***Aspergillus*** & ***Pneumocystis*** infection is suspected

5. see answer and comment 4

6. SDA (Sabouraud Dextrose Agar; basic medium in mycology) is medium with **low pH** - the acidic pH of this medium (pH about 5.0) inhibits the growth of bacteria but permits the growth of yeasts and most filamentous fungi; every clinical specimen from patients with suspected mycosis is seeded on SDA

7. *Madurella* (mold) is the most common cause of fungal **MYCETOMA** (subcutaneous - chronic, pus-producing infection under the skin which sometimes involves bone; the infection most often occurs in the feet); distribution – mainly tropical regions

8. *Candida, Aspergillus, Cryptococcus, Mucorales* (*Mucor, Rhizopus*), *Pneumocystis, Penicillium*

9. **anthropophilic** species of dermatophytes (infections are acquired by contact with infected humans), **zoophilic** species (infections are acquired by contact with infected animals), **geophilic** species (infections are acquired by contact with contaminated soil)

Comment:
Dermatophytes are classified as geophilic, zoophilic, or anthropophilic depending on whether their usual habitat is soil, animals, or humans. Anthropophilic species cause the greatest number of human infections - they elicit relatively mild and chronic infections, produce few conidia in culture, and may be difficult to eradicate. **Zoophilic dermatophytes, being less adapted to human hosts, produce more acute inflmmatory infections - highly inflmmatory reactions that closely resemble pyogenic infections.**

10. Id reaction (autosensitization or autoeczematization) is inflammatory autosensitivity reactions of the skin (like eczema) which occur in association with dermatophytosis but **at sites distant from infection**; this is **immune reaction to hematogenously circulating fungal (dermatophyte) products**; no fungi are identified in the areas of eruption representing the id reaction (culture of skin specimen from Id reaction is negative)

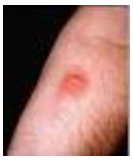
11. 10 - 20% KOH is used for preparing wet preparations

12. *Malassezia* (yeast) **blocks melanin synthesis** in the skin; high humidity and sebum production predispose to infection

13. question is with mistake: it should stand - circle the wrong answer – last one is the wrong answer, so first 4 offered answers are features of *Histoplasma capsulatum*

14. candidosis (=candidiasis)

15. generally, *Candida* skin test (intradermal injection of *Candida* Ag) may be useful in evaluating cellular immune response in patient (assess cellular hypersensitivity to *Candida albicans*); if negative it is suspected that patient has reduced cellular immune response



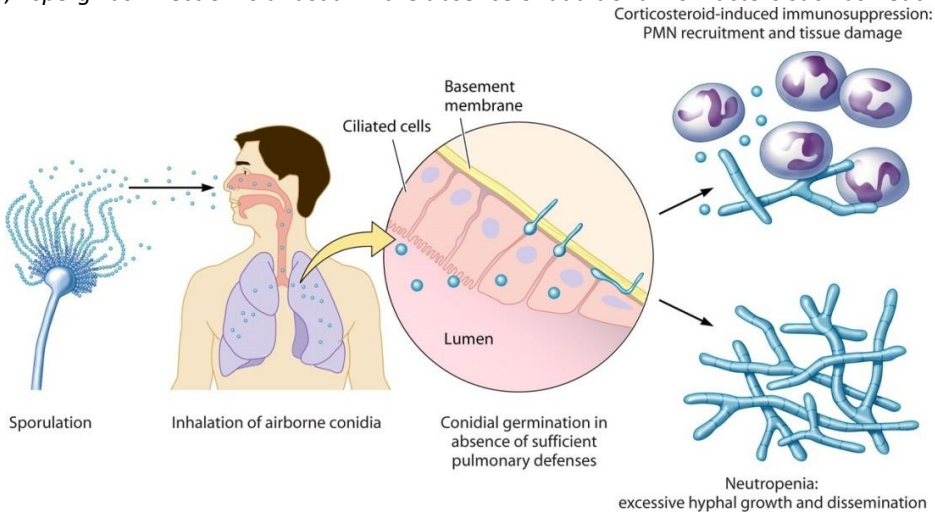
Candida Ag is tested to determine if the individual's immune system is functioning well; a normal immune system demonstrates a positive reaction – ***local swelling and erythema***.

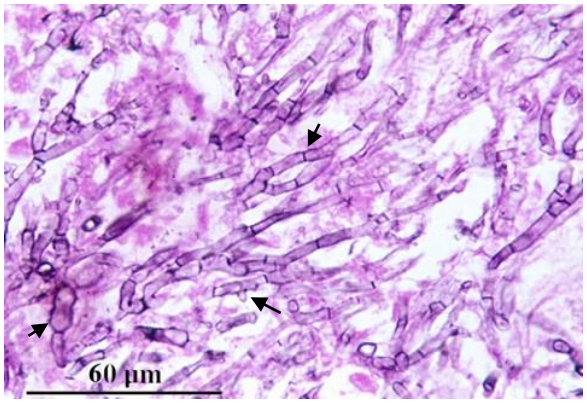
16. yes (oral mucosa, colon, vaginal musosa, in some people on the skin)

17. liver cancer

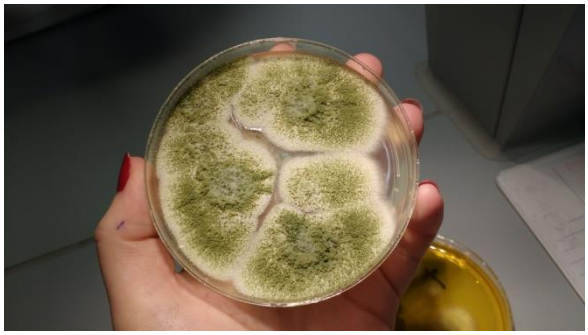
18. the classic and main risk factor for invasive pulmonary aspergillosis is **NEUTROPENIA** (low levels of neutrophils - patients with hematologic malignancy); it is also main risk factor for invasive candidosis

Comment:
Aspergillus is a genus of **mold** that is **ubiquitous in the environment** and can be isolated from soil worldwide. Various species are known to cause human disease but the most frequent pathogen is ***Aspergillus fumigatus***. Human exposure to *Aspergillus* spp. occurs **by inhalation of airborne conidia**. In immunocompetent hosts it is rare for *Aspergillus* to cause disease. In immunocompromised patients or patients with structural lung abnormalities, *Aspergillus* is associated with several distinct syndromes: **aspergilloma**, **allergic bronchopulmonary aspergillosis** & **invasive pulmonary aspergillosis** (IPA). IPA is the most severe and most dangerous manifestation of *Aspergillus* infection and affects **immunocompromised hosts**, particularly patients **with absent or abnormal phagocyte function**. **The risk factors for IPA are corticosteroid use and granulocytopenia from hematologic malignancy or its therapies.** *Aspergillus* syndromes have been described in patients with **HIV infection** but compared to the occurrence of other opportunistic pathogens, ***Aspergillus* infection is uncommon**. Even in patients with very low $CD4^+$ T_H counts, *Aspergillus* infection is unusual in the absence of additional risk factors such as neutropenia.



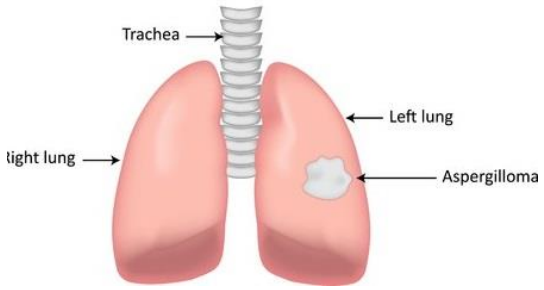


Grocott's methenamine silver (GMS) stained tissue section of lung showing dichotomously branched, septate hyphae of *Aspergillus fumigatus*.



Aspergillus fumigatus (culture on SDA) isolated from lung biopsy specimen.

19. aspergilloma ("fungus ball") is the condition in which accumulation of *Aspergillus* hyphae, cellular debris, and fibrin are present within a pulmonary cavity



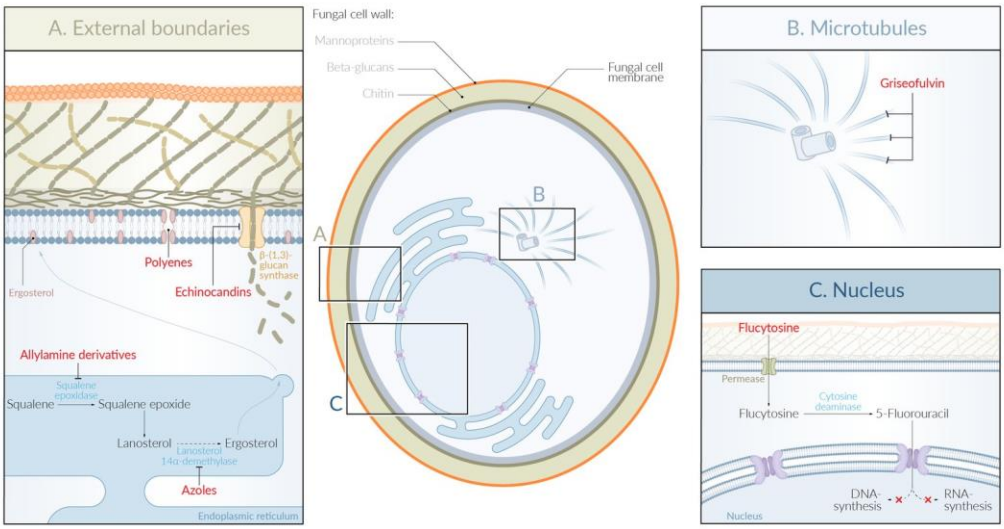
Comment:
Aspergilloma is considered a saprophytic, or noninvasive, form of infection in which ***Aspergillus*** **colonizes preexisting pulmonary cavities caused by tuberculosis**, bronchiectasis, or other conditions. Patients with aspergilloma can be asymptomatic, but many patients have persistent and productive cough, hemoptysis (blood in sputum), and weight loss. Surgical management to extricate the aspergilloma completely is the preferred treatment. Medical management often is unsuccessful because **penetrance of the antifungal agent into the cavity is poor**.

20. no

Comment:
Sometimes *Pneumocystis jirovecii* considered as protozoa, but chemical/genetic analyses showed that is close to fungi. Cell membrane **lacks ergosterol** and hence antifungal agents such as azoles and amphotericin B (drugs that target ergosterol) **are not active against *Pneumocystis***. Cyst cell wall contains **glucans**, which can be helpful for diagnosis and treatment.

21. for *Toxoplasma gondii*; also use for treatment of infections caused by some intestinal protozoa (coccidia and *Blastocystis hominis*)

ADDITIONAL NOTES - ANTIGUNGAL AGENTS (DRUGS) = ANTIMYCOTICS - ANTIMYCOGRAM



Antifungals are used in the treatment of mycotic infections. They are differentiated based on their chemical structure and specific spectrum of efficacy. Some are broad-spectrum antifungals (such as amphotericin B), but some, eg. topical agents (such as clotrimazole) have a limited scope of activity.

ANTIFINGALS EXIBIT DIFFERENT MECHANISMS OF ACTION:

❶ **Binding to ergosterol in the fungal cell membrane** (form pores that disrupt electrolyte balance)

✓ Class **Polyenes** – Amphotericin B, Nystatin

❷ **Inhibition of ergosterol synthesis in the fungal cell membrane**

✓ Class **Azoles*** – Clotrimazole, Miconazole, Ketoconazole, Fluconazole, Voriconazole, Posaconazole,Itraconazole
✓ Class **Allylamines**** – Terbinafine
✓ Class **Morpholines***** - Amorolfine

*inhibit fungal cytochrome P450, which decreases fungal synthesis of ergosterol from lanosterol
** inhibit fungal squalene epoxidase, which decreases ergosterol synthesis
*** inhibit fungal Δ_7–Δ_8 isomerase and Δ_{14}-reductase, which decreases ergosterol synthesis

❸ **Inhibition of β glucan synthesis** (component of the fungal cell wall)

✓ Class **Echinocandins** – Caspofungin, Anidulafungin, Micafungin

❹ **Disruption of fungal mitosis** (bind to keratin and interfere with microtubule function, which disrupts fungal mitosis)

✓ Class **Benzofurans** - Griseofulvin

❺ **Inhibition of DNA/RNA synthesis**

✓ Class **Antimetabolites** – Flucytosine*

* converted to 5-fluorouracil by fungal cytosine deaminase, which then inhibits DNA and RNA synthesis

WHAT IS ANTIMYCOGRAM AND WHY IS IT IMPORTANT

Background

- The increased application of antifungal agents for prophylactic or empirical treatment has led to a change in the epidemiology of fungemia and invasive fungal infections (IFI) and the **emergence of fungal pathogens with decreased susceptibility or resistance to antifungal drugs**
- While *Candida albicans* is the most frequently isolated fungal species in the hospital setting worldwide, **non-albicans *Candida* species (*C. glabrata, C. auris, C. krusei* . . .) with decreased susceptibility to antifungals have emerged as an important cause of IFI**

Antimycogram

- An antimycogram (antibiogram for fungi) is an **examination of the susceptibility of an isolated fungus to antifungals *in vitro*** - shortly **antifungal susceptibility testing**
- In **superficial mucosal mycoses** (eg. oropfaryngeal and vaginal candidosis) **and dermatophytoses** the antimycogram is **rarely used**, except in cases of continuous and recurrent infections (eg. recurrent vulvovaginal candidosis)
- In the case of **IFI** where the fungus is isolated from blood or other sterile fluids and tissues (eg. lung, liver, CSF . . .), an **antimycogram is a mandatory part of laboratory diagnostics** and is necessarily done - the reason for this is that **IFI are often caused by non-albicans *Candida* species (frequently resistant to common antifungals) and molds (*Aspergillus* spp.)**

What methods are used to determine whether a fungus is sensitive or resistant to an antifungal agents?

1. **ANTIMYCOGRAM - PHENOTYPING ASSAYS for Testing Antifungal Susceptibility**
   a. *Conventional methods*
   - **Disk-diffusion method** (use concentration gradients of antifungals that diffuse into growth media)
   - **Broth microdilution method** [measures antifungal activity, expressed as the minimum inhibitory concentration (MIC)* of an antifungal drug which indicates the minimal drug concentration that inhibits fungal growth]
   - **E-test** (allows determination of MIC values)
   - Automated methods

   b. *Nonconventional*
   - MALDI-TOF MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry)
2. **NUCLEIC-ACID BASED ASSAYS FOR DETECTION OF ANTUFUNGAL RESISTANCE – GENETIC ASSAYS (PCR and DNA sequencing)**
   - detection of resistance genes
   - detection of mutations that lead to resistance
   - detection of strains that are sensitive to antifungal agent

***WHAT IS THE USEFULNESS OF THE MIC ?**

The importance of the MIC in the selection of the most appropriate antifungal agent

- **a low MIC** (lower than the susceptible breakpoint) indicates that an antifungal will most likely be effective and is therefore an appropriate choice for treatment
- **a high MIC** (higher than the susceptible breakpoint eg. intermediate or resistant) means that an antimicrobial may have limited or no effectiveness for treatment

The importance of the MIC to define optimal drug dosing regimens

- prevent **underdosing**
- **minimize high dosing** for cost saving

ADDITIONAL NOTES – MUCORALES

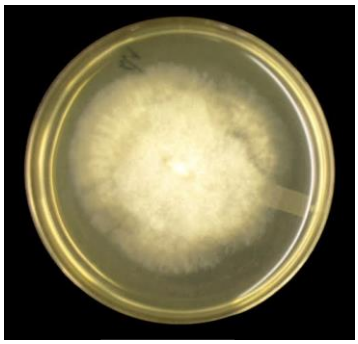
What are Mucorales?

Mucorales are **saprophytic**, aerobic, **ubiquitous molds** in the environment that are commonly found in decaying organic substrates including bread, fruits, vegetable matter, soil, compost piles, and animal excreta. Most important genera of the order *Mucorales* which cause infections in humans are ***Mucor*** and ***Rhizopus***. The disease thay cause is called **mucormycosis which is present worldwide.**

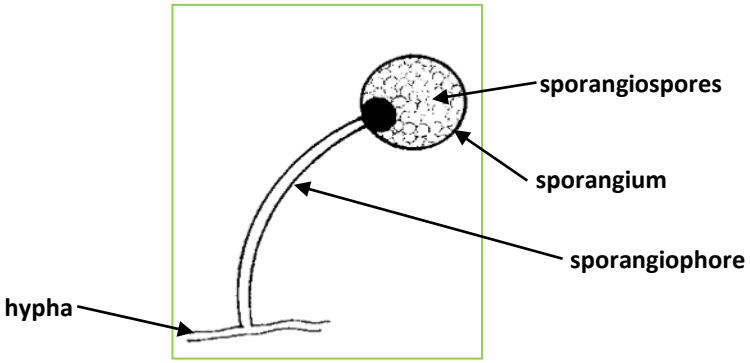
How Mucorales look like?

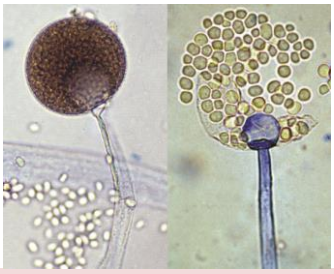
These fungi characteristically produce **large, ribbon-like hyphae** that are irregular in diameter with only occasional septae—hence the characterization of these organisms as **aseptate fungi**. Identification can be confirmed by observing **sporangiophores** with terminal characteristic, saclike structures called **sporangia**, which produce internally spherical yellow or brown **sporangiospores** (asexual spores) (pictures – appearance – drawing and wet prep - in the microscopic preparations from culture).

Colonies are very fast growing, cottony to fluffy, white to yellow or dark-grey in color. *Mucorales* grow at optimal temperature of 30 °C. **Clinical isolates will grow at 37 °C** in the laboratory **2–5 days** after incubation under aerobic conditions.



Culture





How people get infected? What diseases are caused by *Mucorales* fungi?

Spores are easily aerosolized and dispersed and cause infections in humans when **inhaled or introduced through a cutaneous or percutaneous route.** *Mucorales* have a **special predilection for the nasal sinuses and lungs**. **Rhinocerebral and pulmonary mucormycosis** is acquired by inhaling spores. It occurs mainly in diabetic patients and, to a lesser extent, in patients with neutropenia, after transplantation, and in those undergoing steroid treatment. Mucorales have a **high affinity for invading blood vessels** (angioinvasive hyphae; sporangiospores enter the nasal mucosa and paranasal sinuses and invade the carotid and ophthalmic arteries; the invasion causes ischemia, arterial thrombosis, tissue infarction, and necrosis).

How to diagnose mucormycosis?

Sensitivity of culture is poor. Diagnosis is usually made by **histopathology**, visualizing the characteristic hyphae invading tissue in samples obtained from **biopsy**. If culture remains negative, further identification of the species with **PCR** on the histology sample can be used.