



14



# Microbiology

Doctor 2018 | Medicine | JU

● Sheet

○ Slides

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Well hello there, long time no see! Hope you're up for some fun, because this sheet's theme is party theme!! With our special guest, the (fun-guy)!! So, are you ready to have some fun with our fun-guy?? Well, maybe not for you guys who say fun-jai but anyway you're also invited 😊.

This sheet's topics are: characteristics of fungi, classification, diseases, diagnosis, and antifungal treatment.

**Medical mycology** is the medical field specialized in studying mycoses (diseases caused by fungi) of humans and their etiologic agents (causes).

### \*\*Fun facts about fungi:

- There are more than 300,000 types of fungi, those of medical importance are less than 300, and 90% of fungal infections are caused by only a few dozens.
- Fungal invasion of human tissue was recognized in the early 1800s before the science of bacteriology was developed.
- They can help us make food, (obviously party food). e.g. baked-goods like cake (yeast), wine, beer, and cheese. But never eat peanuts in fun-guys parties; (see fungal toxins p6)

### ❖ Now let's get to know fungi more closely:

1. They're harmless, lazy creatures coming from the kingdom of fungi.

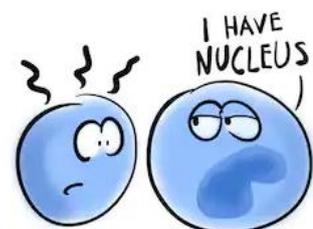


Mycologic infections are often harmless; they heal spontaneously, with some exceptions of course, especially in immunocompromised people who get exposed to opportunistic fungi.

Fun-guys are lazy, they're like these spoiled people who don't like working and only wanna eat (decompose organic compounds) and party (cause infection), it needs time for them to get things done → **the presentation of fungi is indolent (subacute), it needs time for the disease to occur.** (they always show up late).

2. They're eukaryotes.

They have a true nucleus, DNA in chromosomes, an endoplasmic reticulum, Golgi apparatus and mitochondria.



### 3. They reproduce by budding or by spores, sexually and asexually.

Spores, which are reproductive units (don't get them confused with bacterial spores of survival). They're very important, because sometimes when we want to describe fungi under the microscope, we might refer to the morphology of the spores, and they can be pathognomonic. (specific for a certain type of fungi). (See last page).

### 4. All fungi require organic source of Carbon associated with decaying matter (heterotrophs).

Fungi are abundant in nature, they're called **major decomposers** because they decompose organic material, so they are of two types:

- a. **Saprophytic**; decomposes organic material on dead cells (for Halloween parties).
- b. **Parasitic**; decomposes organic material on living tissue. (live shows).

### 5. Most fungi are obligatory aerobes, some are facultative aerobes. But there are NO obligate anaerobes, or else how are they gonna come to our party?

#### ❖ **Fungal cell:**

Since they have their own kingdom, Fungi have certain **characteristics that distinguish them from:**

#### 1. **Eukaryotes:**

- a. **The plant kingdom**, fungi lack **chloroplasts** -chlorophyll.
- b. **The animal kingdom**, fungi have **cell wall**. Also, the plasma membrane in fungi contains **ergosterol**, whereas in humans it's cholesterol.

#### 2. **Prokaryotes:**

fungi's cell wall **lacks peptidoglycans**. Rather, it's made of **polysaccharides** that gives it the rigidity.

Having a rigid cell wall is a feature that have been taken advantage of in the lab for separating fungi from human tissue.

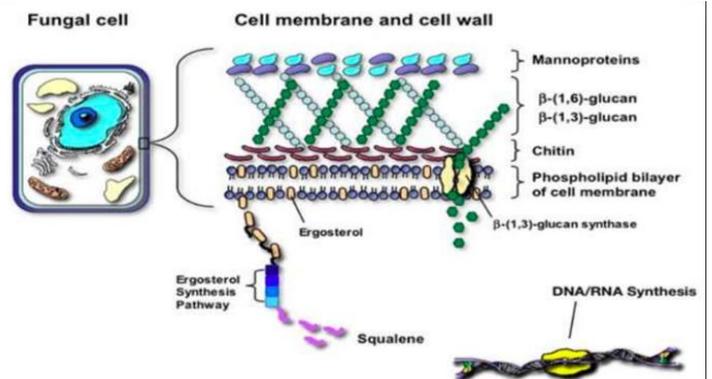
**i.e. when obtaining fungi samples, we use the strong alkaline potassium hydroxide (KOH) to digest the human tissue, but the fungi will still be there because of the rigidity of the cell wall. Thus, we're able to separate these fungi from human tissue.**

\*\* These unique characters the fungi possess are important in medications, especially antifungals, because we can target them without harming the human tissue.

The cell wall and the enzymes synthesizing ergosterol are good targets for antifungals.

**Fungal cell wall:** consists of a group of polysaccharides, these include: chitin,  $\beta$ -glucan, and mannans. The cell wall is antiphagocytic.

**Fungal plasma membrane:** consists of phospholipids and **ergosterol**.

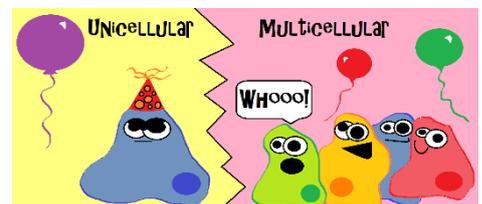


### ❖ Importance of fungi:

1. They are common cause of damage to crops and food chain. (some are party crashers).
2. They can be used in the pharmaceutical industry (antibiotics) (e.g. penicillin from penicillium fungi) and enzymes.
3. Production of food.
4. They establish increasing infections especially in immunocompromised patients.

### ❖ Fungi are classified according to their morphology into three groups:

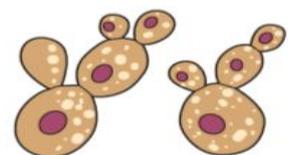
1. Yeasts (single cell)
2. Molds; filamentous hyphae (singled cell or more)
3. Dimorphic, fungi that can grow as yeasts and as molds under certain conditions



Mushrooms can be considered as a group. However, these are not of medical importance unless they contain a toxin.

### 1. **Yeasts:** these are the babies (single cell), they love candy! (candida).

- oval or round shaped
- Reproduce by **budding**; daughter cell arises from the mother cell as a tiny bud separated by a narrowing called isthmus. This is important for you to remember that the mother cell is not of equal size to the daughter cell.
- Could form pseudohyphae



Please differentiate between division and budding. Division results in equally sized cells.

- **Examples of yeasts (very important):** *Candida albicans* and *Creptococcus neoformans*.

*Candida albicans* can be a yeast, pseudohyphae, and hyphae. Which gives it the term **polymorphic** fungi. *Candida* could alter its shape under certain environmental conditions. (candy comes in different shapes doesn't it?).

Pseudohyphae; a result of incomplete budding where the cells elongate but remain attached.



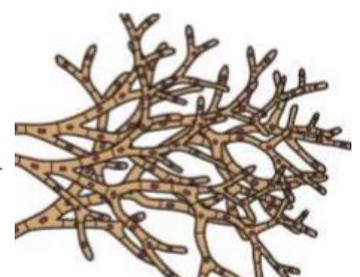
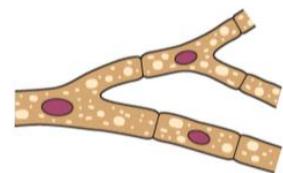
*Candida* is part of the normal flora in skin, GI, and vagina. It can cause infections in immunocompromised patients (it's opportunistic).

Immunocompromised: can be due to diseases like AIDS, side effect of drugs like corticosteroids and chemotherapy of cancers, drugs used by patients following allograft (immune-suppressors), or even pregnant women

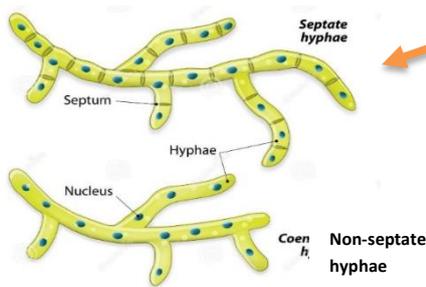
- Deep fungal infections are related to immunocompromised patients. *Candida* can cause meningitis, arthritis and respiratory infections.
- Unlike *Candida albicans*, *Cryptococcus neoformans* are exogenous -always pathogenic- they come from soil and pigeons' feces, and they have a capsule. They can infect us (if immunocompromised) through spores, inhalation, or accidental ingestion – be careful of what you eat when you go to fun-guys parties and keep you distance from them or you might go home with a lung infection.

## 2. Filamentous hyphae (molds):

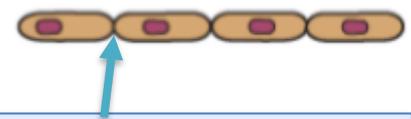
- They have tubular filaments (hyphae) which may be septate or non-septate.
- They're always branched.
- Mono or multicellular
- Branched, Intertwined hyphae are called **mycelium**; mat like structure
- Reproduce by asexual **spores** at the tip or side of the hyphae. Asexual spores may be contained in a sac called sporangiospores.



## Septate and non-septate??



Cells here are separated by septa; partitions, unlike pseudohyphae.



These are NOT septa, they are constrictions. We cannot classify it if it has septa or not, because it is pseudohyphae

- **Examples include (very important):** Zygomycetes, Aspergillus and Dermatophytes.
  - a. **Zygomycete:** it causes a disease called mucormycosis. It has 3 species: Rhizopus, mucor, and Absidia.
  - b. **Aspergillus,** includes three species:
    - a. aspergillus fumigatus, related to fungal allergies
    - b. aspergillus flavus
    - c. aspergillus parasiticus

The last two can cause **Aspergillosis**; an infection, usually of the lungs, it forms a ball of fungus fibers, blood clots, and white blood cells. People may have no symptoms or may cough up blood or have a fever, chest pain, and difficulty breathing. (piñata!)

- c. **Dermatophytes (ring-worm):** dermatophytosis is a common skin infection (Tinea diseases). They have three species:
  - a. Epidermophyton
  - b. Microsporum
  - c. Trichophyton

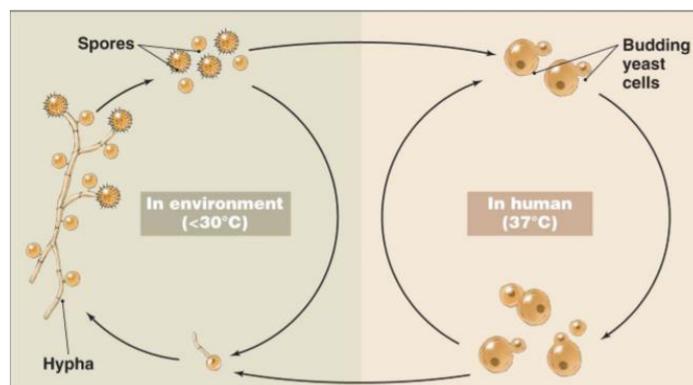
### 3. Dimorphic fungi:

- These occur in two forms: yeast form in tissues or when grown at 37°C & filamentous form when grown at 22°C. And this is actually considered a key virulence factor. (they don't like paparazzi, they put on disguise when going out).
- The conversion between the two forms is reversible, and if inhibited, the fungi will not be parasitic anymore (it can't grow in the human host).
- **Examples (very important):**
  - a. Blastomyces dermatitidis.
  - b. Coccidioides immitis, causes a disease called coccidioidomycosis
  - c. Histoplasma capsulatum, causes histoplasmosis
  - d. Paracoccidioides, causes Paracoccidioidomycosis

- They cause **endemic** (infect healthy people in certain areas of the world), also called **systemic** (multiple systems) infections.

This is how dimorphism happens, the temperature is the key factor.

Remember that it's yeast at 37°C, can be either in human tissue or in the lab (incubation).



## ❖ Fungal diseases:

Fungal infections have recently emerged as a growing threat to human health, especially to people whose immune systems are compromised in some way.

### 1. Fungal allergies

- Molds grow on any damp organic surface, and spores are constantly in the air.
- Inhaled Spores & volatile fungal toxins may play a role in producing allergic manifestations such as **asthmatic reaction** (rapid Broncho-constriction mediated by IgE) and eosinophilia.
- Notable in **Aspergillus fumigatus**
- Occupation related; commonly seen in farmers.

#### Examples:

- a. **Extrinsic allergic alveolitis**; refers to a group of lung diseases in which your lungs become inflamed as an **allergic** reaction.
- b. **Allergic rhinitis**; also known as hay fever, a type of inflammation in the nose which occurs when the immune system overreacts to **allergens** in the air.

### 2. Fungal toxins (mycotoxicosis):

- One of the main group of toxins is **Aflatoxin**; a group of structurally related toxic compounds produced by certain strains of fungi (*Aspergillus flavus* & *Aspergillus parasiticus*).
- **Aflatoxicosis** is a poisoning condition & it results from ingestion of aflatoxins in aflatoxins contaminated food.
- Under favorable conditions of temperature & humidity, these fungi grow on certain foods & results in production of aflatoxins.

- The most pronounced contamination has been encountered in tree nuts, peanuts & other oilseeds including corn. (that's why peanuts are not allowed in our party)
- Aflatoxins are metabolized in the liver to epoxide, which is a potent carcinogen.
- Aflatoxin B1 is the most naturally occurring, potent hepatocellular carcinogen. It induces mutation in the p53 human suppressor gene, leading to loss of growth control in hepatocytes.
- It leads to crops and plants damage.

### 3. Fungal infections (mycoses): (most important)

- Fungal infections range from superficial infections to overwhelming infections that are rapidly fatal in compromised host.
- Classified to: superficial, cutaneous, subcutaneous, opportunistic, and endemics.
- The infection with fungi is increasing in frequency as a result of increased use of antibiotics, corticosteroids & cytotoxic drugs (immunosuppression).

#### a. Superficial & cutaneous:

- ✓ Infections involve the skin (dead, keratinized layer), mucous membranes, nail or hair.
- ✓ **Superficial:** **without** tissue destruction or immunological reaction.  
e.g. pityriasis versicolor (by *Malassezia furfur* complex), Tinea nigra, white piedra, and black piedra.
- ✓ **Cutaneous:** minor pathological changes due to the presence of the fungi or its metabolites.  
e.g. cutaneous candidiasis & dermatophytes.
- ✓ (they like to party on the roof, so the house remains clean inside)

#### b. Subcutaneous

- ✓ Infection is confined to sub-cutaneous tissue (cornea, muscles, and joints), but without dissemination to distant organs. (they party indoors, but with soundproof walls -they don't disturb the neighbors).
- ✓ All the subcutaneous diseases must penetrate the epithelial barrier, that's why it's called **traumatic implantation**; the fungi enter the body through minor cuts on skin.
- ✓ **Examples:**
  1. Chromoblastomycosis
  2. *Sporothrix Schenckii* (**very important, the doctor said it's an exam question**), it causes sporotrichosis, also called **rose gardener disease**.

There's a proverb that says: "he who wants a rose, must respect the thorn". And that's the case here. Housewives and gardeners who get skin cuts and abrasions from thorns are at risk of getting infected by *sporothrix schenckii*, which lives in soil, hay, and rose bushes. Effects are most commonly seen as nodules on the arms.

### c. Systemic (endemic) mycoses

- ✓ They occur in certain geographic areas.
- ✓ They're caused by dimorphic fungi.
- ✓ Most important thing to know is that they cause diseases in healthy people.
- ✓ Are primarily pulmonary lesion that may disseminate to any organ. (affects multiple system in the body).
- ✓ These are wild parties, but they throw parties in specific countries only.

e.g. coccidioidomycosis, histoplasmosis.

### d. Opportunistic Mycoses

e.g. Candida species (normal flora), Cryptococcus (exogenous).

## ❖ Diagnosis

Diagnosis of fungal infections is based on a combination of clinical observation and laboratory investigation.

### Clinical investigation

The first indication that a patient may have a **systemic mycosis**, is often their failure to respond to antibacterial antibiotics .

### Laboratory diagnosis

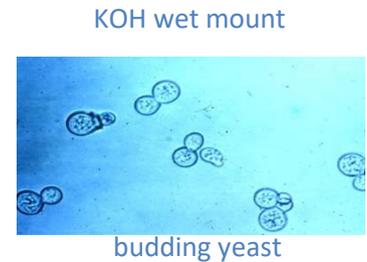
- ★ Recognition of the pathogen in tissue by **microscopy**
- ★ Isolation of the causal fungus in **culture**
- ★ The use of **serological tests**
- ★ Detection of fungal **DNA** by PCR (culture may be slow; this method is faster but more expensive)

## ❖ Types of specimen

- Skin scales, nail clippings and scrapings of the scalp that include hair stubs are the most suitable specimens for the diagnosis of ringworm, or **skin infections** in general. These are collected into folded paper squares for transport to the laboratory .
- For **subcutaneous infections** the most suitable specimens are scrapings and crusts, aspirated pus (fluid) and biopsies (tissues).
- Swabs should be taken from suspected Candida infections from the **mucous membranes** and preferably sent to the laboratory in 'clear' transport medium.
- In suspected **systemic infection**, specimens should be taken from appropriate sites.

## ❖ Stains and Direct Microscopic Examination

- Most specimens can be examined satisfactorily in wet mounts after partial digestion of the tissue with 10–20% **potassium hydroxide**. (The KOH is enough, but it can be stained for further specificity).



- Addition of Calcofluor white (universal stain) and subsequent examination by fluorescence microscopy enhances the detection of most fungi as the fluorescent hydroxide– Calcofluor binds to the fungal cell walls (chitin). (seen under UV light).
- special stains:

India ink; to stain cryptococcus neoformans, as it stains the outer capsule.

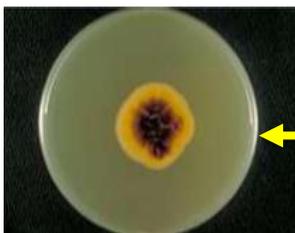
Others: methylene blue, lactophenol blue, periodic acid-Schiff (PAS), ink, gram stain.

## ❖ Culture

Most pathogenic fungi are easy to grow in culture.

Sabouraud dextrose medium (SDA agar): [\(see figures below\)](#)

- Commonly used because it inhibits bacterial growth (except for contaminants).
- May be supplemented with chloramphenicol (antibiotic) to minimize bacterial contamination and cycloheximide (antifungal) to reduce contamination with **saprophytic** fungi (may come from the flora of the person who’s handling the specimen).



This is dermatophytes culture; yellow border with a black crust center.

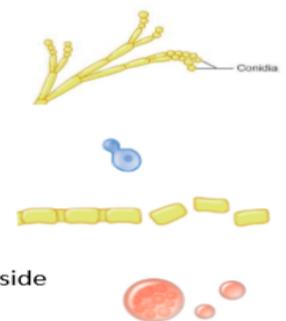
This is a typical Candida culture, it has a special colony shape you should keep it in mind; it’s white and has a creamy texture.



This slide is extra; it’s just for us to understand these terms when they come up in the next lecture.

### Spore types

- Conidial Asexual spores | Formed off of hyphae | Common | Airborne
- Blastoconidia: “Buds” on yeasts (asexual budding daughter yeast cells)
- Arthroconidia: Asexual spores formed by a “joint”
- Spherules and Endospores (Coccidioides): Spores inside the spherules in tissues



## ❖ Antifungal therapy

- The drugs used to treat bacterial diseases have no effect on fungal infection (mainly because the cell wall doesn't have peptidoglycans).
- The problem with antifungals is their narrow therapeutic window -high toxicity. That's why oral antifungals are prescribed once weekly.
- People who are on oral antifungals for long periods of time, or who take it IV should be tested for liver functions regularly.
- It depends on the presence of ergosterol in fungal cell membranes.

### Classified into six groups:

Type of antifungal	Mechanism of action	examples
<b>Polyenes derivatives</b>	<u>The only Fungicidal</u> ; will KILL the fungi. They would bind the membrane ergosterol → disrupt the plasma membrane of fungi.	<b>Amphotericin B</b> → given IV in endemic mycoses or deep opportunistic mycosis. <b>Nystatin</b> → topical
<b>Azoles</b>	<u>Fungistatic</u> ; inhibit the growth without killing. They inhibit the synthesis of ergosterol	<b>Ketoconazole</b> <b>Fluconazole</b> <b>Itraconazole</b> <b>Voriconazole</b> <b>Posaconazole</b>
<b>Griseofulvin</b> (Extracted from fungi)	Fungistatic	Prescription is NOT common
<b>5-flouorocytosine (5-FC)</b>	Fungistatic , Inhibitor of DNA and RNA synthesis	
<b>Allylamines</b>	<u>Fungistatic</u> . Inhibit the enzyme for ergosterol synthesis; squalene epoxidase.	<b>Terbinafine (Lamasil)</b> Commonly prescribed
<b>Echonocandins</b> For dermatophytosis (superficial and cutaneous)	<u>Fungistatic</u> . Disrupt the cell wall by inhibiting β-glucans synthesis (1,3-β-glucan synthase).	<b>Caspofungin</b>

Well, it's already midnight and the fun-guy better get going, or it might lose its magical party spirit.

Thank you so much for bearing and I hope you have good day. ❤️