

Doctor 021

# MICROBIOLOGY

Sheet no. 12



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# INTRODUCTION TO MYCOLOGY

**Medical mycology is the study of mycoses of man and their etiologic agents.**

Medical mycology is the study of infections that affect susceptible humans.

**Mycoses are diseases caused by fungi. Of the several thousand species of fungi known, less than 300 are pathogenic to man.**

-In **fungal diseases**, they call it **Mycoses** or **Mycotic diseases**. They have 3 forms: under allergies, under toxins, and fungal infections. (collectively known as fungal diseases).

-The number of species that cause diseases in humans is less than 300. From these 300, a few (dozen) cause 90% of fungal diseases.

**fungal invasion of human tissue was recognized in the early 1800s before the science of bacteriology was developed.**

-Manifestation of fungal infections vary, not specific to fungal diseases (we will talk about it more later..). Highly dependent on high index of susceptibility.

-The main difference between **fungi** and others (bacteria, viruses...) is that there is **no acute presentation**. Their presentation (manifestation) tends to be: Subacute (indolent), Subclinical, and insidious.

-A lot of these diseases are harmless, most of the patients see a doctor (you) for cosmetic diseases (more than pathology or immune presentation or have signs and symptoms).

## WHAT IS A FUNGUS?

### Kingdom fungi

**A-** When we talk about fungi we talk about **Eukaryotics**, which means **atrue** (well-defined) **nucleus**, Golgi apparatus, ER, Ribosomes.... (totally different than prokaryotes).

They are at higher complexity than prokaryotes.

**B-** They are different from plants as they **Do not contain chlorophyll** (Photosynthetic energy-producing mechanism).

Don't mix them with algae.

**C- Heterotrophic; means that they gain their carbon or nitrogen source from a. decaying matter (dead tissue) known as Saprophytic They gain nitrogen from ammonia and nitrate. or**

**b. living organism (in case of fungal diseases) known as Parasitic (biotrophs).**

**D-** there are morphological forms of fungi: **yeast** (single cell), multicellular **filamentous structures** (tubular **hyphae**) which we call **molds**. (Also mushrooms).

**E- Produce spores (sexual & asexual reproduction).**

Spores (here): reproducing elements (not something that has to do with survival and resisting like bacteria).

when they're producing sexually they are teleomorphic fungi, Asexually called anamorphic fungi.

Spores and conidia interchangeably: conidia imply Asexual spores.

(some relevant spores: arthrospores, ascospores, vacuospores, blastospores, sporules حزوخذهم الموحاضرة الجاي نكالفش)

**F- The cell wall consist of chitin and B-glucan, both are polysaccharide which is the site of action of some antifungal drugs.** some works specifically on the cell wall in glucan synthesis like echinocandins (has a drug named caspobiem).

**G-** They are different from animals as **they have ergosterol in their cell membrane** (while animals have cholesterol). And it is the site of action of some antifungals such as Polyenes, Azoles, and Allyamines. (works on the biosynthesis of ergosterol).

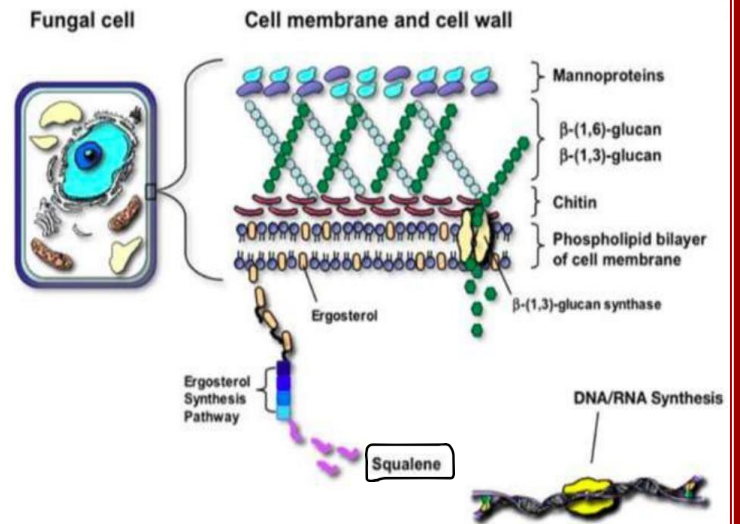
**H- Most fungi are obligatory aerobes** and some are facultative anaerobes but never obligatory anaerobes.

## Fungal Cell:

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.



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.

Allylamine works in squalene epoxidase at earlier stages of ergosterol synthesis.

## ❖ Importance of fungi:

**1- They are a common cause of damage to crops and the food chain.**

**2- They can be used in the pharmaceutical industry (antibiotics) (e.g. penicillin from penicillium fungi), Enzymes, single-cell proteins..**

**3- Production of food. Like bread, cheese, beer....etc.**

**4- Few species of fungi can cause disease in human (300/200,000). However, fungal infections are increasing due to AIDS and other immunosuppressant conditions.**

They establish increasing infections, especially in immunocompromised patients. you give a patient antibiotics and don't respond, first thing pops up is that it is a fungal infection. debilitated more prone to develop fungal infections we call them **opportunistic infections**).

Ex :: IV  $\rightarrow$  mucormycosis (zygomycosis). may develop in diabetic patients.

# GENERAL MYCOLOGY

• Fungi can be classified morphologically and according to growth forms into:

1- Yeast (single cell)

2- Molds; filamentous hyphae (single cell or more)

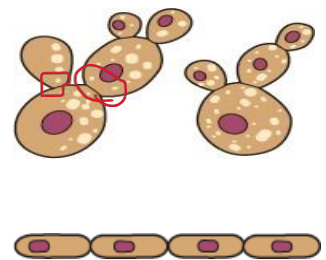
3- Dimorphic, fungi that can grow as yeasts and as molds under certain conditions

❖ **Yeast:** these are the babies (single cell), they love candy! (candida)

➤ **oval or round shaped**

➤ **Reproduce by budding not division** (in budding daughter cell are smaller than mother cell while in division have equal sizes).

-Between them, there is constriction, not septation.



➤ **May form pseudohyphae (chains of elongated budding cells)**(result of incomplete budding where the cells elongate but remain attached).

➤ **Examples of yeasts: Candida albicans , Creptococcus neoformans. both opportunistic organisms**

a- **Candida** (endogenous) is part of the normal flora in the skin, GI, and vagina. It can cause infections in immunocompromised patients (it's opportunistic, once you get immunocompromised you develop candidiasis) a lot entity presentations, like AIDs have oral thrush. Cutaneous candidiasis leads to candida creases (armpit).

**Deep fungal infections are related to immunocompromised patients. Candida can cause meningitis, arthritis and respiratory infections.**

Candida can be exogenous only in sexual contact.

Females can develop vaginal indeniasis.

b- cryptococcus neoformans exogenous -always pathogenic- they come from soil and pigeons' feces (cryptococcus diseases) **it is not a part of normal flora.**

**it commonly infects lung initially.** and they have a capsule.

They can infect us (if immunocompromised) through spores, inhalation, or accidental ingestion.

❖ **Filamentous fungi ( Molds):**

➤ **They have branching tubular filaments (hyphae ) which may be septate or nonseptate**

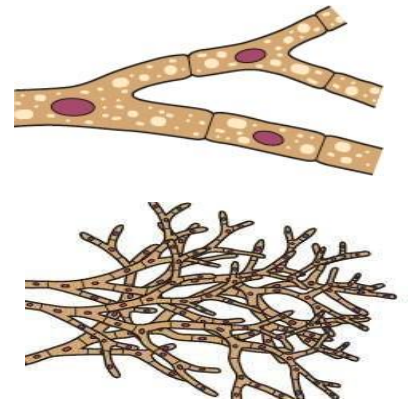
➤ -They're always branched. -Mono or multicellular

➤ **Mycelium: mass of branching, interlinking hyphae ,** mat like structure.

➤ **Also may produce asexual spores at the tip or side of the hyphae**

**they can result in fungal infection or allergy when they are inhaled They are located inside spherules called sporangiospores**

➤ **e.g Zygomycetes, Aspergillus and Dermatophytes**



**a. Zygomycete:** it causes a disease called mucormycosis.

It has 3 species: Rhizopus, mucor, and Absidia.

**Aspergilloma in lung could be respective to conidia of aspergillus**

**b. Aspergillus induces all fungal disease and infections,** includes three species:

1. aspergillus fumigatus, related to fungal allergies
2. Aspergillus flavus
3. 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

**c. Dermatophytes (ring-worm):** common skin infection (Tinea diseases).

**They have three species:**

- a. Epidermophyton      b. Microsporum      c. Trichophyton

❖ **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 disguises when going out).

➤ The conversion between the two forms is reversible (**thermal sensitive**), and if inhibited, the fungi will not be parasitic anymore (it can't grow in the human host). **parasitic form of dimorphic fungi is yeast**

➤ **Examples:**

**a. Blastomyces dermatitidis. blastomycosis**

**b. Coccidioides immitis,** causes a disease called coccidioidomycosis.

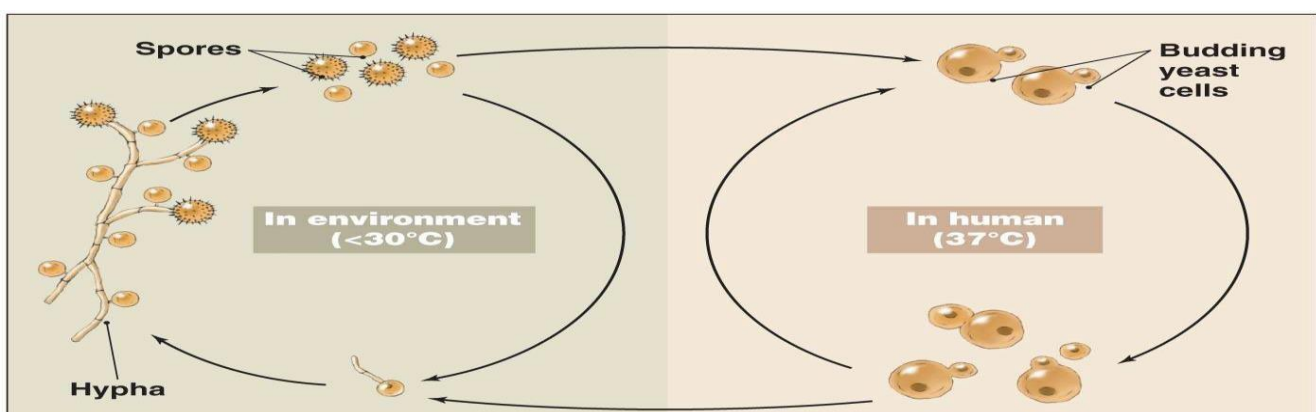
**c. Histoplasma capsulatum,** causes histoplasmosis

**d. Paracoccidioides,** causes Paracoccidioidomycosis.

**e. Sporothrix, cause sporotrichosis (rose gardeners disease)**

➤ They cause endemic (infect healthy people in certain areas of the world), also called systemic (multiple systems) infections. **there density in the three continents of America. they differ from opportunistic fungi as they are true pathogens (they can cause disease even in immunocompetent patients)**

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 bronchoconstriction mediated by IgE) and eosinophilia.**
- **Notable in *Aspergillus fumigatus***
- **Occupation-related; commonly seen in farmers. *fungi conidia* (spores) induce hypersensitive reaction type 1**
  
- **Examples:**
  - 1- Extrinsic allergic alveolitis; refers to a group of lung diseases in which your lungs become inflamed as an allergic reaction.
  - 2- 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
  - 3- Asthma (bronchoconstriction in bronchial tree)

### 2. Fungal toxins (mycotoxicosis): One of the main groups is Aflatoxin.

- **Aflatoxicosis is a poisoning condition & it results from ingestion of aflatoxins in aflatoxins contaminated food.**
- **Aflatoxins are group of structurally related toxic compounds produced by certain strains of fungi ( *Aspergillus flavus* & *A.parasiticus*)**
- **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.
- 
- Aflatoxins are metabolized in the liver to epoxide, which is potent carcinogenic. **Mushrooms also produce toxins called Amanitin such as LSD**
- 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. **Mutation in p53 gene can result in overgrowth of hepatocytes in certain step in cell cycle**
- 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).

❖ Human fungal infections are commonly classified as:

#### a. Superficial & cutaneous:

Infections involve the skin (dead, keratinized layer), mucous membranes, nail or hair.

- **Superficial:** without tissue destruction or immunological reaction. e.g. **(tinea) pityriasis versicolor (by Malassezia furfur complex), Tinea nigra, white piedra, and black piedra.**

The most outer layer is stratum corneum. Patients complain from cosmetic appearance and they do not show signs or symptoms from the

infection.

- **Cutaneous:** minor pathological changes due to the presence of the fungi or its metabolites. e.g. cutaneous candidiasis (appear between fingers due to frequent washing hands) & dermatophytes. such as: Tinea capitis: scalp hair, tinea corporis in body like: tinea cruris in thigh creases and tinea pedis in leg (between toes)

### **b. Subcutaneous:**

**infection is confined to sub-cutaneous tissue (cornea, muscles, and joints), but without dissemination to distant organs.**

All 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 caused by *Fonsecaea pedrosoi***

2. **Sporothrix schenckii**: it causes sporotrichosis, also called rose gardener disease.

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.

3. **Madura foot caused by madural mycecomatis**

### **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).**

e.g. *coccidioidomycosis*, *histoplasmosis*. *paracoccidioidomycosis*, *blastomycosis*.

### **d. Opportunistic Mycoses:**

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

they have very low inhaled virulence (usually harmless)

## ❖ 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:**

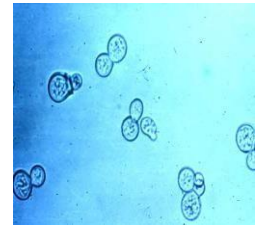
- Recognition of the pathogen in tissue by microscopy
- Isolation of the causal fungus in culture (the most specific and sensitive mean
- The use of serological tests
- detection of fungal DNA by PCR

## ❖ 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).



KOH wet mount budding yeast

**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:**

**Commonly used** because it inhibits bacterial growth (except for contaminants).

### **May be supplemented with:**

**a-chloramphenicol** (antibiotic) **to minimize bacterial contamination**

**b-cycloheximide** (antifungal) **to reduce contamination with saprophytic fungi** (may come from the flora of the person who's handling the specimen).



Candida culture

it has a special colony shape

you should keep it in mind

it's white and has a creamy texture.



dermatophytes culture

yellow border with a black crust center.

❖ **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.

Amphotericin B and nystatin are polyenes (Fungicidal: killer drug) & various azoles (fungistatic: inhibitor of growth) are commonly used for treatment of fungal infection.

Classified into six groups:

Type of antifungal	Mechanism of action	examples
<b>Polyenes derivatives</b>	The only <b>Fungicidal</b> ; 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> (الكيتونك يا زول) <b>Fluconazole</b> <b>Itraconazole</b> <b>Voriconazole</b> <b>Posaconazole</b>
<b>Griseofulvin</b> (Extracted from fungi) <b>penicillium</b>	Fungistatic <b>Given for superficial mycotic</b>	Prescription is NOT common
<b>5-flouorocytosine (5-FC) F</b>	Fungistatic , Inhibitor of DNA and RNA synthesis	<b>Chemotherapeutic agent given to cancer</b>
<b>Allylamines</b>	<u>Fungistatic</u> . Inhibit the enzyme for ergosterol synthesis; squalene epoxidase.	Terbinafine (Lamasil) 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).	Caspofungin

# V2

Any thing written in orange is new -><-

# V3

Page 8, “Aflatoxins are group of structurally related toxic compounds produced by certain strains of fungi ( *Aspergillus flavus* & *A.parasiticus*)”, has been added.