Sheet 4

INTRODUCTION TO MYCOLOGY

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Introduction To Mycology

We're going to venture into a different area of microbiology, exploring the fascinating world of mycology, which delves into the study of fungi.

Medical Mycology

Medical mycology is the study of mycoses of man and their etiologic agents.
It is a branch of medical science that focuses on studying fungal infections, also known as mycotic infections. It involves understanding the causes, symptoms, and treatments of this type of infection.

- Mycoses are diseases caused by fungi. Of the several thousands of species of fungi that are known, less than 300 are pathogenic to man. Among these, roughly a dozen species is responsible for 90% of all fungal infections in humans.
- Fungal invasion of human tissue was recognized in the early 1800s before the science of bacteriology was developed.

What is a Fungus?

Kingdom Fungi
Eukaryotic – a true nucleus, heterotrophic, do not contain chlorophyll

- Fungi are eukaryotic organisms with distinct features that differentiate them from prokaryotes. They possess organelles such as the endoplasmic reticulum (ER) and Golgi apparatus, as well as 80S ribosomes instead of the 70S ribosomes found in prokaryotes.
- Fungi require an external (exogenous) source of organic material for their nutrition, making them heterotrophs. They are considered major decomposers in ecosystems because they break down organic material to obtain carbon for their metabolic needs.

Two morphological forms:
Yeasts الخميرة & filamentous structures (hyphae) العفن

Yeasts are unicellular fungi, whereas hyphae are multicellular structures. Hyphae are characterized by their tubular shape, which forms the filamentous network known as mycelium in many fungi.

Produce spores (sexual & asexual reproduction)

1) Unlike bacterial spores, which are formed to protect bacterial cells from harsh conditions, fungal spores serve as reproductive structures and are not involved in resistance.

2) Fungal multiplication is complex, occurring through both asexual and/or sexual reproduction:
   - Asexual spores: Include *conidia*, *arthrospores, blastospores and chlamydospores*, and are associated with anamorphs (pure asexual reproduction).
   - Sexual spores: Include *ascospores, basidiospores and zygospores*, and are associated with teleomorphs (pure sexual reproduction).

3) The term "spores" is often used interchangeably to refer to both sexual and asexual forms of reproduction in fungi.

🔔 What is in gray was declared unimportant by the professor during the lecture.

Saprophytic (on dead tissue) vs Parasitic (on living organisms).

- Saprophytic: Obtain nutrients from dead or decaying organic material. The majority of fungi fall into this category and play a vital role in decomposing organic matter.
- Parasitic (Biotrophic): Derive nutrients from living organisms, often causing harm to their host. This category includes fungi that infect humans.

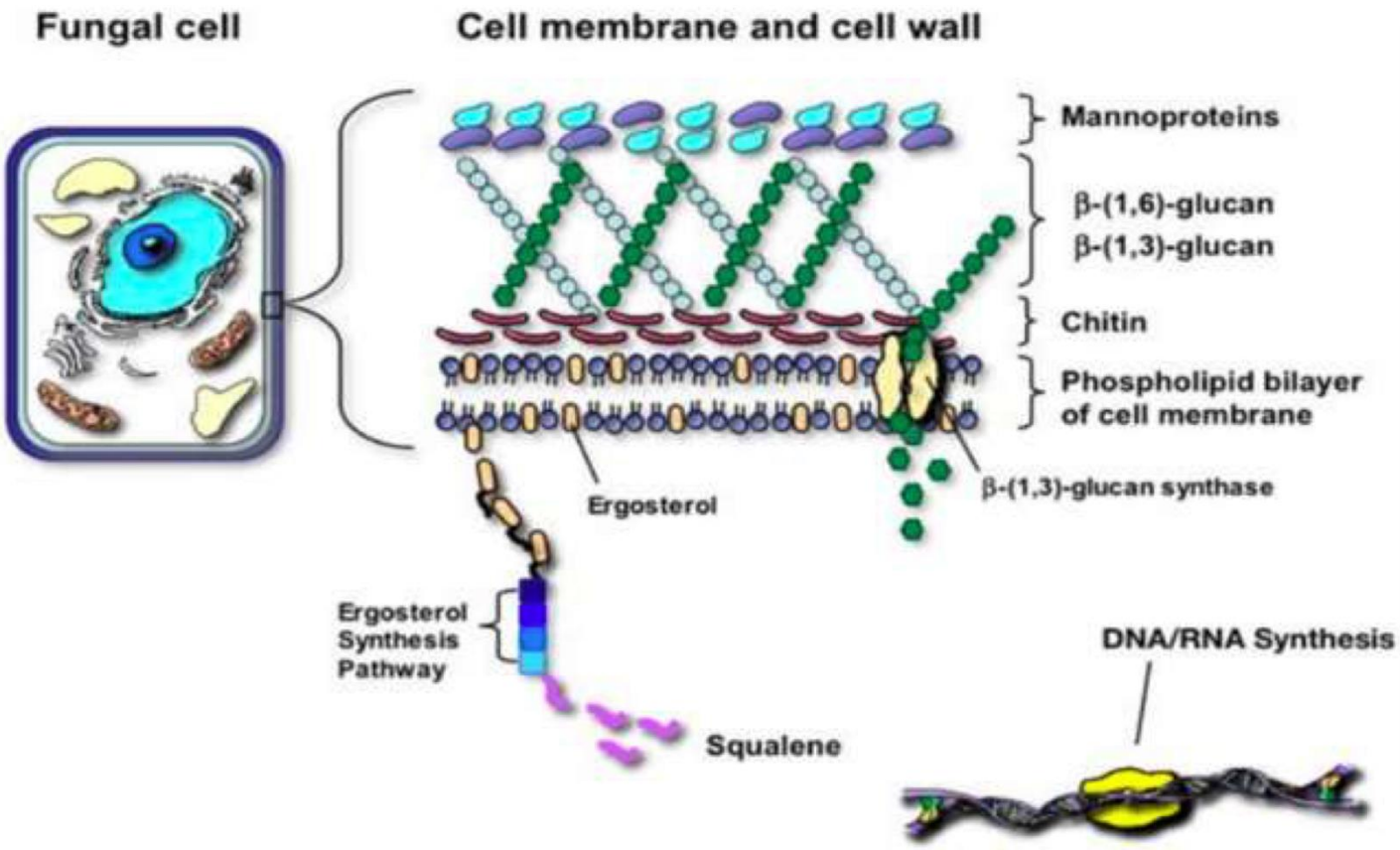
All fungi require organic sources of carbon associated with decaying matter.

Cell wall consists of chitin and B-glucan, both are polysaccharides, which are the sites of action of some antifungal drugs.

- Fungal cell walls are composed of polysaccharides, primarily chitin, rather than peptidoglycan, which is found in bacterial cell walls. Fungal cell walls also contain glucans and mannans.
- Chitin plays a crucial role in the investigation of mycotic infections, as it can be utilized in the universal protocol for fungal identification. A 10–20% solution of potassium hydroxide (KOH), a strong alkalinizing agent, is commonly used. KOH dissolves all components of the sample except for fungi, as their chitin-rich cell walls resist disintegration.

Cell membrane consists of ergosterol instead of cholesterol. Ergosterol is the site of action of some antifungal drugs.

- Most Fungi are obligate aerobes.
- Some are facultative anaerobes.
- None are obligate anaerobes.



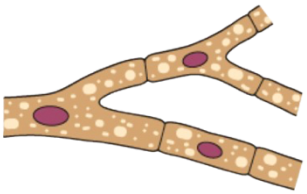
Interpretation of the Illustration:

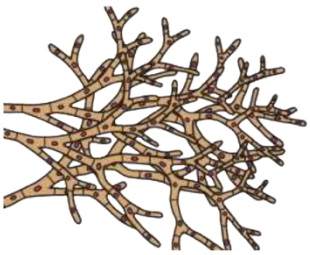
- This schematic diagram represents a fungal cell, which is a eukaryotic organism. It has a true nucleus, and its cytoplasm contains organelles such as ribosomes, the Golgi apparatus, and the endoplasmic reticulum (ER). The fungal cell wall is composed primarily of chitin, glucans, and mannans, which are the major structural components of fungal cells.

- The plasma membrane of fungal cells is rich in ergosterol, a sterol unique to fungi. Antifungal drugs can target different parts of the fungal cell:
  1. Some disrupt the plasma membrane.
  2. Others interfere with the cell wall.
  3. Certain antifungals inhibit squalene epoxidase, an enzyme involved in one of the earliest steps of ergosterol synthesis before it is incorporated into the fungal plasma membrane.

The Importance of Fungi:

- Food Industry: Used in bakery processes, such as bread-making, and in yogurt fermentation.
- Industrial Applications: Play a key role in the production of citric acid and various enzymes.
- Beverage Production: Essential for fermenting products like wine, beer, and other alcoholic beverages.
- They are common causes of damage to crops and the food chain, often causing significant crop spoilage and loss of agricultural yield.
- Few species of fungi can cause disease in humans (300/200,000). However, fungal infections are increasing due to AIDS and other immunosuppressant conditions.
- Production of antibiotics e.g. Penicillin.





General Mycology

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

1. Yeast:

- Unicellular
- These are oval or round cells that reproduce by budding (asexual)
- The daughter cell formed during fungal reproduction is smaller in size compared to the mother cell.
- May either form pseudohyphae (chains of elongated budding cells) or separate from the mother cell.
- Pseudohyphae are not tubular and lack true septation. Instead, they exhibit constrictions at their junctions.

- *Candida albicans* and *Cryptococcus* neoformans
  - **Candida**: Some *Candida* species are commensal organisms and are part of the normal flora in humans. However, in immunocompromised patients, they can cause multisystem infections, such as meningitis, arthritis and respiratory infections, collectively referred to as candidiasis.
  - **Cryptococcus neoformans**: This species is always pathogenic when present. It is commonly found in soil and pigeon feces, posing an occupational hazard to individuals working with pigeons. *Cryptococcus neoformans* commonly infect the lungs initially, causing an exogenous infection known as cryptococcosis, a potentially severe fungal infection usually involving the CNS.

2. Filamentous Fungi (Molds/Hyphae):

- Multicellular
  - They have branching tubular filaments (hyphae) which may be septated or non-septated (septated forms have true septa unlike pseudohyphae seen in some yeasts).
  - Mycelium: mass of branching, interlinking hyphae.
    When fungal hyphae overlap and interconnect, they form a dense, mat-like structure known as mycelium.
  - The body of mycelium is called thallus.
- Also, may produce asexual spores at the tip or side of the hyphae.
- Asexual spores may be contained in a sac called sporangiospores.
- E.g. *Zygomycetes* (Mucorales), *Aspergillus* (Aspergillosis) and *dermatophytes* (ring worms).

- *Zygomycetes*: *Rhizopus, Abisidia* and *Mucor.*
- *Dermatophytes*: *Trichophyton, Epidermophyton* and *Microsporum.*

3. Dimorphic Fungi:

- They claim both morphological forms, but only one form inside the human body.

- These fungi exhibit two forms:

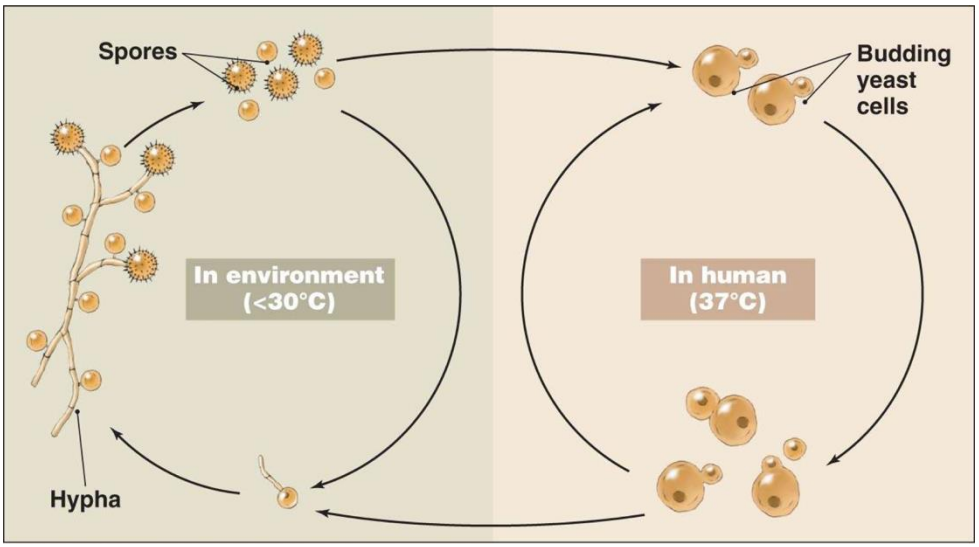
1. Yeast form: Found in tissues inside the human body or when grown at 37°C.
2. Filamentous (hyphae/mold) form: Found in the environment or when grown at 22°C.

- The morphological change in dimorphic fungi is triggered by environmental factors, primarily temperature, and is reversible. This characteristic is used in laboratory diagnosis.

- Examples (endemic mycotic diseases) include:

1. *Blastomyces dermatitidis* - Blastomycoses
2. *Coccidioides immitis* - Coccidioidomycosis
3. *Histoplasma capsulatum* - Histoplasmosis
4. *Paracoccidioides brasiliensis*
5. *Sporothrix schenckii*

- These fungi are pathogenic and can cause disease in immunocompetent individuals. In immunocompromised individuals, infections are more severe. Their distribution is geographically limited.
- Some infections, such as Candida, exhibit yeast, pseudohyphae, and true hyphae forms within the human body. In contrast, dimorphic fungi exist in only one morphological form at a time, depending on whether they are in the environment or inside the human body.



Fungal diseases:

- Remember, there is another less medically important type of fungi: mushrooms.
- Fungal infections have recently emerged as a growing threat to human health, especially to persons whose immune systems are compromised in some way.

There are other forms of fungal diseases apart from fungal infections (mycoses), such as fungal allergies and mycotoxicosis:

1) Fungal allergies:

Molds grow on any damp organic surface, and spores are constantly in the air.

Inhaled Spores, conidia & 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*, can cause external allergic rhinitis. Simple manifestations usually include rhinorrhea, and more advanced cases include asthma.

2) Fungal toxins (mycotoxicosis):

Aflatoxicosis:

Aflatoxicosis is a poisoning condition & it results from ingestion of aflatoxins in contaminated food.

Aflatoxins are a group of structurally related toxic compounds produced by certain strains of fungi **(*Aspergillus flavus* & *A. parasiticus*).**

- Aflatoxins are considered mutagens and carcinogens as well as immunosuppressants.

Under favorable conditions of temperature & humidity, these fungi grow on certain foods (fruits/vegetables) & resulting 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.

Aflatoxin B1 (most well-known toxin) induces mutation in the p53 human suppressor gene (and suppresses this tumor suppressor gene), leading to loss of growth control in hepatocytes, potentially causing hepatocellular carcinoma.

- Apart from *Aspergillus* spp., *Amantia* mushrooms are also toxic, as well as mushrooms used for the extraction of certain drugs such as LSD mind-altering drug.

3) Fungal infections (mycoses):

Fungal infections are the most encountered entity of fungal diseases.

Fungal infections range from superficial infections to overwhelming infections that are rapidly fatal in compromised host.

- There are 5 classifications of fungal infections: Superficial, cutaneous, subcutaneous, systemic and opportunistic fungal infections.
- We fear subcutaneous, systemic and opportunistic mycosis. Also, the status, severity and type of fungal infections depend on the host, for example, immunocompromised patients are more likely to develop systemic mycotic diseases.
- Systemic mycotic diseases have poor prognosis (bad outcomes)

The infection with fungi is increasing in frequency as a result of increased use of antibiotics, corticosteroids & cytotoxic drugs (immunosuppression).

- The number of immunocompromised people has increased in the last 2 decades; and that is due to many factors such as: immunosuppressants or broad-spectrum antibiotics.
- This noticeably increases the risk for mycotic infections.

Human fungal infections are commonly classified (clinically based on the anatomical site of the mycotic infection) as:

Superficial & cutaneous infections:

Infections involve the skin, mucous membrane, nail or hair with or without tissue destruction & immunological reaction.

- Superficial mycosis for example infects only the stratum corneum of the skin.
- Cutaneous mycosis involves slightly deeper structures and affects nails and hair.

- **Superficial mycosis** involves no living tissue and is thus characterized by no tissue damage and no immunological reaction which means that superficial mycosis has limited symptoms.

e.g. pityriasis versicolor, Tinea nigra & black and white Piedra.

- Symptoms are usually confined to color changes, either hypo- or hyperpigmentation (depending on the base-line color of the patient's skin). No itchiness or other symptoms are typically noticed in superficial mycosis.

- **Cutaneous mycosis** is actually associated with tissue damage and immunological reaction, with relatively more noticeable and prominent symptoms such as inflammation and pain. Itchiness of the site is typical of cutaneous mycosis.

e.g. cutaneous candidiasis & dermatophytes.

- Dermatophytes are also called ringworms (not to be confused with helminths). The reason for this naming is the ring-shaped lesions caused by this type of infection.

Subcutaneous infections:

In subcutaneous fungal infections, the infected layer is deeper than the layers infected by superficial and cutaneous fungal infections.
Examples of the layers (subcutaneous layers) infected by subcutaneous fungal infection: fascia, cornea of the eye and muscles.

Now, the question is how would the fungus reach these subcutaneous layers?
Such infections are usually caused after **traumatic implantation** through the skin.

Infection is confined to sub-cutaneous tissue without dissemination to distant organs.

Examples of subcutaneous fungal infections:

- Chromoblastomycosis ➔ chronic granulomatous infection affecting legs and arms.
- Sporotrichosis ➔ which is caused by the dimorphic fungi *Sporothrix schenckii*.

Sporotrichoisis gets into the body after splinter trauma by rose splinters affecting garderners or housewives when dealing with roses thus called “rose gardener disease”.

Sporotrichosis can affect alcoholics, but this type is pulmonary and not subcutaneous.

- Eumycetoma or Madura foot (caused by *Madurella mycetomatous*) usually by farmers walking barefooted on infected soil.

Opportunistic mycosis:

The individual must be immunocompromised so that opportunistic fungi take the opportunity to cause opportunistic mycotic diseases, they are not necessarily fromthe normal flora.

Examples on fungi which cause opportunistic mycosis:

1) *Candida spp*. ➔ part of the normal flora ➔ causes endogenousinfection.
2) *Cryptococcus* ➔ always pathogenic not microbiota ➔ causes exogenous infection.
3) *Aspergillus* ➔ rhinorrhea or severe asthma or even lung lesions (aspergillomas).
4) *Pneumocystis jirovecii* ➔ mainly infecting patients with AIDS.
5) *Zygomycetes* (Mucorales) ➔ mainly infect diabetic patients with ketoacidosis.

Systemic mycosis:

Are primarily pulmonary lesions that may disseminate to any organ.

The individual doesn't have to be immunocompromised to be infected by those diseases as it also infects healthy individuals. However, if the individual was immunocompromised, the systemic mycotic disease would be more severe withmore complications.

Most Systemic mycotic diseases are caused by **dimorphic fungi**, they are characterized by having certain geographical distributions, as they are considered endemic in certain areas of the world, but not in our area.

Examples of systemic mycotic diseases:

1) Coccidioidomycosis ➔ caused by *Coccidioides immitis*
2) Histoplasmosis ➔ caused by *Histoplasma capsulatum*
3) Blastomycosis ➔ caused by *Blastomyces dermatitidis*
4) Paracoccidioidomycosis ➔ caused by *Paracoccidioides brasiliensis*

Notice that the examples above are dimorphic fungi → systemic mycosis.

So, we have 5 main types of mycosis (mainly divided according to anatomic site):

1) Superficial:
   Confined to the stratum corneum with no inflammation, mainly depigmentation.

2) Cutaneous:
   Slightly deeper with live tissue damage and hair and nails involved, symptomatic.

3) Subcutaneous:
   Traumatic implantation of the fungi through the outer layer into deeper sites.

4) Opportunistic:
   Occurs due to immunodeficiency (cancer, AIDS, corticosteroids, etc.)

5) Systemic:
   Primary pulmonary lesions that disseminate (mainly caused by dimorphic fungi).

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.

Generally, fungi which cause superficial and cutaneous infections are relatively easier to diagnose because of the symptoms seen by the physician on the patient's skin.

Other types of mycoses are more challenging to identify and need further investigation and laboratory diagnosis.

Laboratory diagnosis:

1. Recognition of the pathogen in tissue by microscopy.
2. Isolation of the causal fungus in culture **(most sensitive way).**
3. The use of serological tests (to detect the presence of a fungal antigen by the presence of specific antibodies against this antigen).
4. Detection of fungal DNA by PCR.

   Specific probes are famously used to detect fungal antigen of *cryptococcus neoformans* in the CSF of the patient suspected to have cryptococcosis.

These techniques are required if the infection is not superficial or cutaneous, such as being subcutaneous or systemic.

Types of specimen

Skin scales, nail clippings and scrapings of the scalp that include hair stubs and skinscales are the most suitable specimens for the diagnosis of ringworm; these are collected into folded paper squares for transport to the laboratory.

- Nails are used as specimens in the infection involves the nails. Same for the hair.
- Skin scales can be used in superficial mycoses such as pityriasis versicolor.

Swabs should be taken from suspected *Candida* infections from the mucous membranes and preferably sent to the laboratory in 'clear' transport medium.

For subcutaneous infections the most suitable specimens are scrapings and crusts, aspirated pus and biopsies.

So, in subcutaneous mycotic infections we need other methods to obtain the specimen, the most suitable approaches are biopsy and aspirate.

The difference between biopsy and aspirate:

- **Biopsy**: solid with fluid
- **Aspirate**: fluid only

In suspected systemic infection, specimens should be taken from appropriate sites.

If there was an infected organ, then the process of taking the specimen depends on the involved organ.

Stains and Direct Microscopic Examination

Directly searching for fungal cells in specimens is possible yet not so specific.

There is a universal approach for the diagnosis of fungal infections:

- Most specimens can be examined satisfactorily in wet mounts after partial digestion of the tissue with 10–20% potassium hydroxide.

We first obtain the specimen suspected of having a fungal infection, then we put in in a 10%-20% Potassium hydroxide, which is a very strong alkalinizing agent that disintegrates everything in the specimen apart from fungal cells because of their resistant chitin.

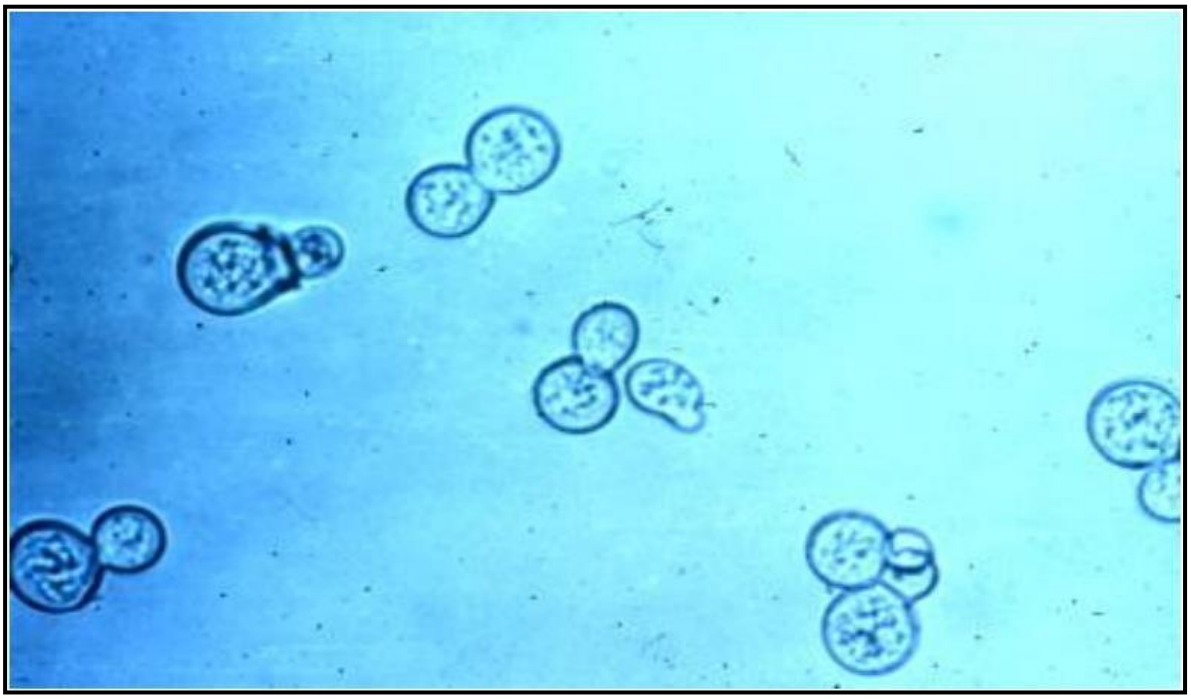
- The addition of Calcofluor white and subsequent examination by fluorescence microscopy enhances the detection of most fungi as the fluorescent hydroxide–Calcofluor binds to the fungal cell walls.

Calcofluor white (universal stain) can be added for extra details of the fungal morphology.

- Special stains (methylene blue, lactophenol blue, periodic acid-Schiff (PAS), ink, etc.)

Those are used occasionally. Gram stain can also be used. *Candida* is gram positive.

KOH wet mount; clear background due to killing of all cells except fungi.



Notice the budding yeasts.

Culture

After treating with KOH and detecting fungal infection, culture can be done.
Most pathogenic fungi are easy to grow in culture (they can grow on bacterial agarplates).

Sabouraud dextrose medium:

- commonly used (universal agar for culturing).

- may be supplemented with:
  1. Chloramphenicol ➔ to minimize bacterial contamination
  2. Cycloheximide ➔ to reduce contamination with saprophytic fungi (fungi present in the environment, which is non-pathogenic).

It is a customized agar plate for the growth of pathogenic fungal species.

Normal bacterial culture agars can be used, but keep in mind that fungi are usually slower in growth in comparison with bacteria.

These bacterial agars can be customized for pathogenic fungal growth by adding chloramphenicol or cycloheximide.

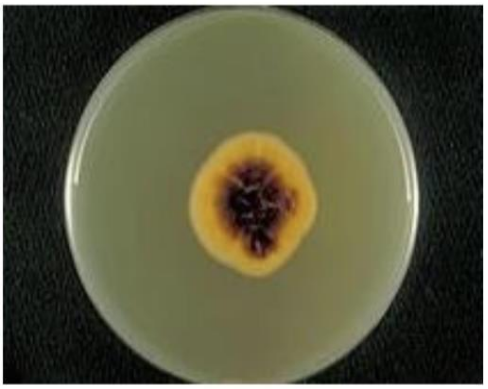
The top figure shows *Candida albicans* culture.

It is described as white, blubberous, creamy, waxy colonies.

On Sabouraud dextrose agar (SDA).

The bottom figure shows Dermatophytes culture.

Also on Sabouraud dextrose agar.



Antifungal therapy

The drugs used to treat bacterial diseases have no effect on fungal infections.

- Antifungals have narrow therapeutic windows and have severe ***adverse*** effects.
- There are 6 main families of antifungals (see next).
- Allylamines, azoles, and polyene derivatives are the 3 most used ones.

It depends on the presence of ergosterol in fungal cell membranes.

✓ Amphotericin B and nystatin are polyenes (fungicidal);
✓ and Various azoles (fungistatic) are commonly used for treatment of fungal infection.

Fungicidal → Kills Fungistatic → Inhibits the growth

These are the **6 groups** of antifungals for the treatment of fungal diseases:

1) **Polyene derivatives** (The only fungicidal group of antifungals)

- Amphotericin B ➔ systemic member which is given IV.
- Nystatin ➔ oral, ointment and cream.

All the remaining antifungals (5 groups) are fungistatic (they inhibit the growth of fungi rather than killing them directly).

2) **Azoles** (The most famous fungistatic group of antifungals)

- Ketoconazole
- Fluconazole
- Itraconazole
- Voriconazole
- Posaconazole

Azoles are Present in many forms, topically and IV.

They interfere with the pathway of ergosterol synthesis during the synthesis process and not after formation. This is why they are fungistatic and not fungicidal.

3) Griseofulvin

- Unique mechanism; binds to microtubules.

4) 5-fluorocytosine (5-FC)

- Cytosine analogue.
- They are very potent DNA and RNA replication inhibitors.
- They are used in cancer treatment.

5) Allylamines

e.g., Terbinafine (Lamasil)

- Commonly used drug.
- They inhibit the enzyme squalene epoxidase which is involved in the early stages ofergosterol biosynthesis.

6) Echinocandins

e.g., Caspofungin & Micafungin

- Inhibits the synthesis of glucans in the cell wall.

Allylamines, azoles and polyenes are the ones you will most probably have access to as a GP (general practitioner).

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