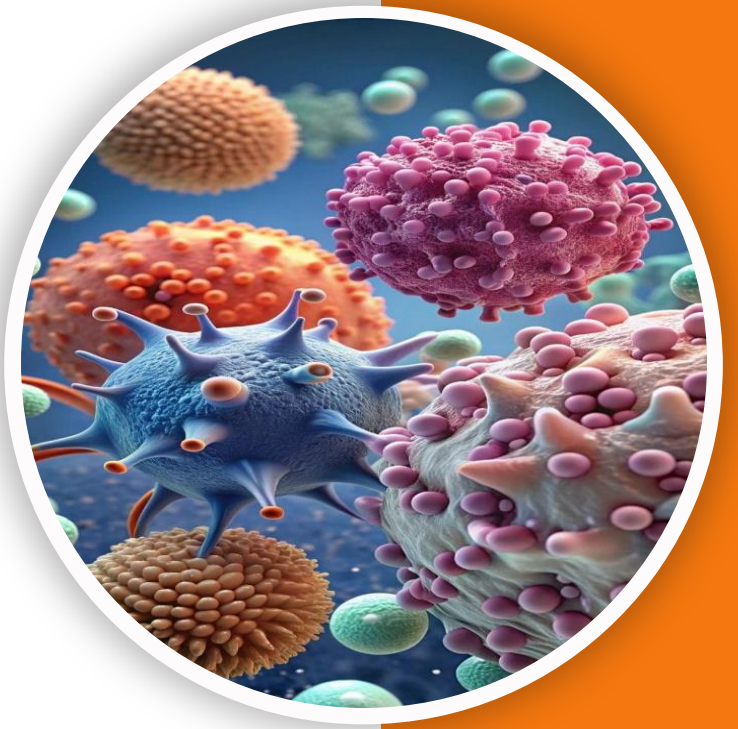
بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Microbiology | Lecture 1

Introduction To Mycology



Written by : Jannat Al-Ahmad
Rawan Okour

Reviewed by : Maram Darweesh

Introduction to Mycology

By: Nader Alaridah

Medical mycology

is the study of mycoses of man and their etiologic agents.

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

Among them, about 10 to 20 species account for 90% of all human fungal infections.

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

This is why we put fungi in a separate kingdom from plants

- **Yeasts &** filamentous structures (**hyphae**)
- Produce spores (sexual & asexual reproduction)
- Saprophytic (on dead tissue).Parasitic (on living organism).
- All fungi required organic source of Carbon associated with decaying matter

Eukaryotic

Fungi are eukaryotic organisms characterized by features that set them apart from prokaryotes. They contain organelles like the endoplasmic reticulum (ER) and Golgi apparatus, and have 80S ribosomes rather than the 70S ribosomes present in prokaryotic cells.

Yeasts & filamentous structures (hyphae) العفن

Fungi exhibit two morphological forms:
yeasts, which are unicellular,
hyphae, which consist of multicellular filaments.

Produce spores (sexual & asexual reproduction)

1.Unlike bacterial spores, which form to protect bacterial cells from harsh conditions, fungal spores function as reproductive structures and do not play a role in resistance.

2.Fungal multiplication is complex:
Asexual spores: Include conidia, also called anamorphs (pure asexual reproduction).
Sexual spores: Also called teleomorphs (pure sexual reproduction).

3.The term “spores” is often used to describe both sexual and asexual reproductive forms in fungi.

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

Saprophytic fungi obtain nutrients from decaying organic matter, acting as decomposers, whereas biotrophic (parasitic) fungi derive their nourishment from living organisms, often causing disease in their hosts.

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

They are considered major decomposers in ecosystems, breaking down **decaying organic material** to obtain **carbon** for their metabolic needs, while depending on **ammonium nitrate** as their main **nitrogen** source.

- **Cell wall** consist of **chitin** and B-glucan ,both are polysaccharide which is the site of action of some antifungal drugs.

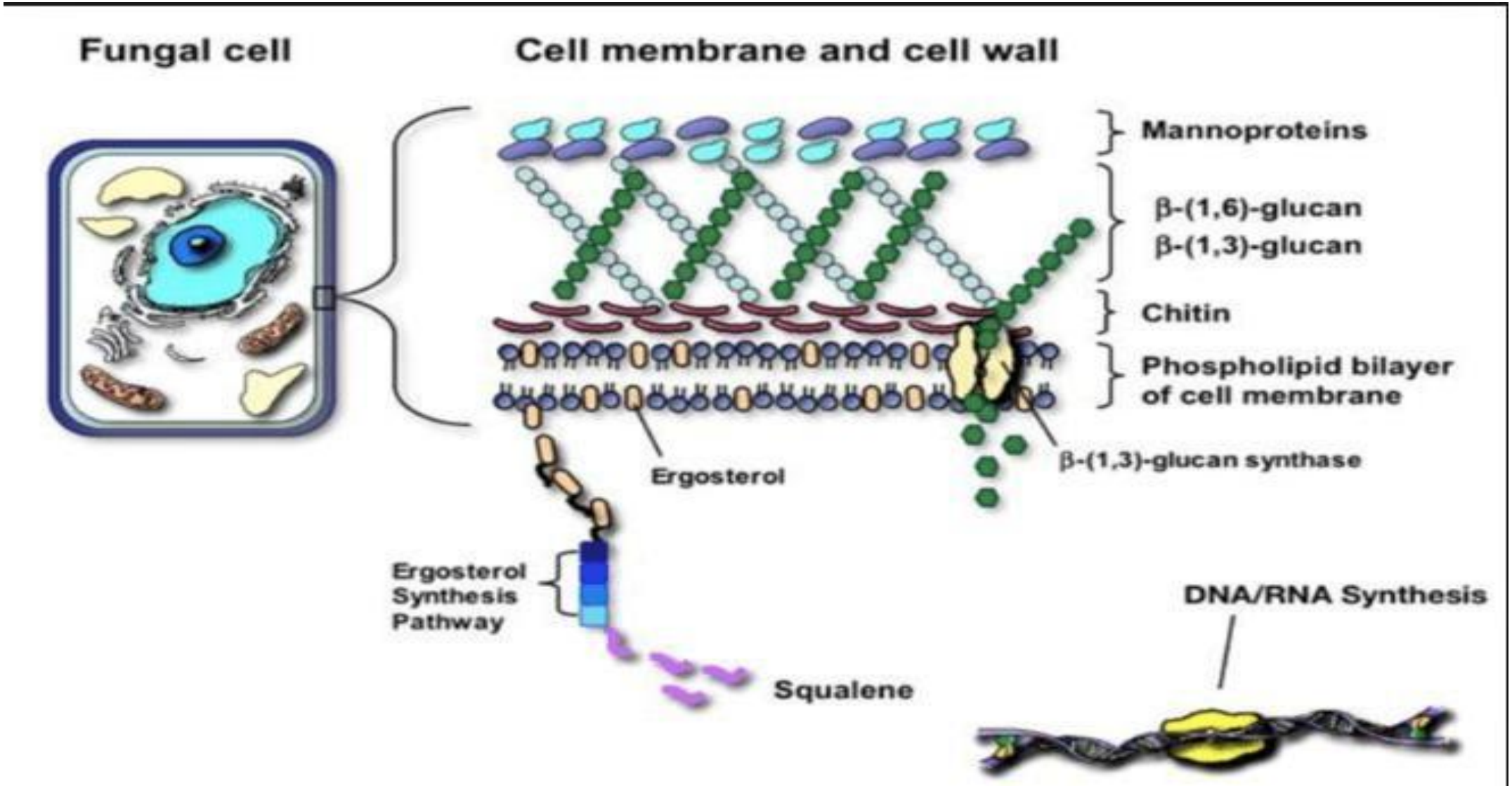
- **Cell membrane** consist of ergosterol Ergosterol is the site of action of some antifungal.

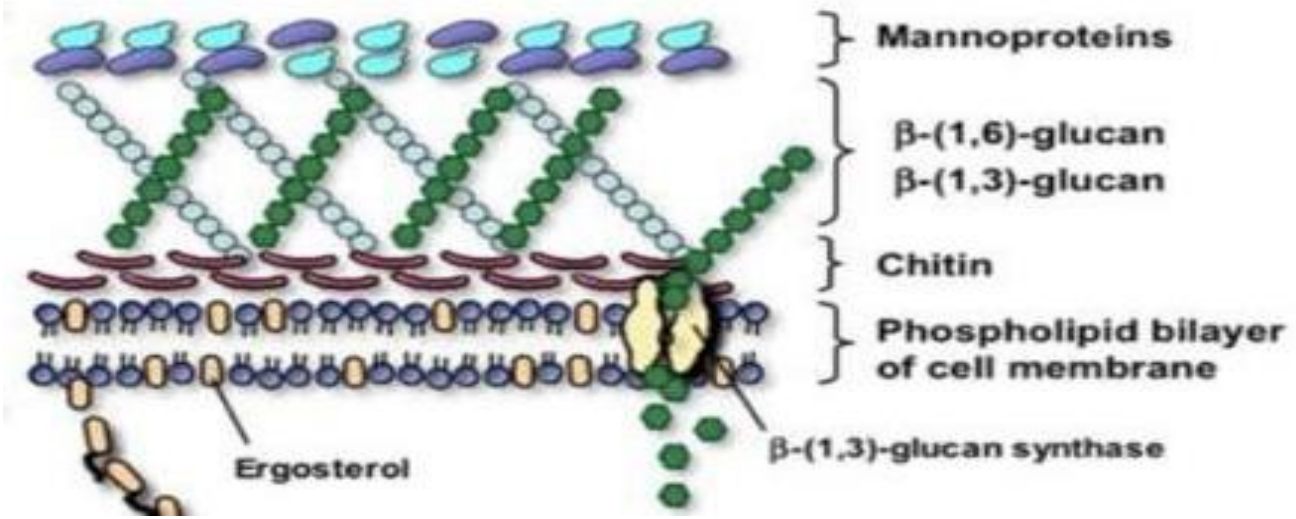
- Most fungi are obligatory aerobes . They rely on oxygen for their respiration

  Some are facultative anaerobes. → They are ordinarily aerobes but under certain condition they rely on fermentation rather than respiration

  None are obligate anaerobes.

Fungal Cell


Fungal cell
Cell membrane and cell wall
Mannoproteins
β-(1,6)-glucan
β-(1,3)-glucan
Chitin
Phospholipid bilayer of cell membrane
Ergosterol
β-(1,3)-glucan synthase
Ergosterol Synthesis Pathway
Squalene
DNA/RNA Synthesis



Fungal cell walls are composed of glucans, chitin and mannans.

Chitin is important in diagnosing mycotic infections because it can be targeted in general protocols for fungal identification.
A 10–20% potassium hydroxide (KOH) solution, a strong alkaline agent, is commonly used. KOH dissolves all components of a sample except the fungi, whose chitin-rich cell walls remain intact.

Cell membrane consists of ergosterol instead of cholesterol.

Ergosterol is the site of action of some antifungal drugs.

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)**Azoles** – inhibit enzymes involved in ergosterol synthesis, disrupting the plasma membrane.

2)**Polyenes** – bind directly to ergosterol in the membrane, causing cell leakage and death.

3)**Allylamines** – inhibit squalene epoxidase, an enzyme in an early step of ergosterol synthesis before it is incorporated into the plasma membrane.

The Importance of Fungi

1 They are common cause of damage to crops and food chain.

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

3. Production of antibiotics e.g Penicillin.

Additional Uses

Food Industry: Used in bakery processes like bread-making and in yogurt fermentation.

Industrial Applications: Important for producing citric acid and enzymes.

Beverage Production: Essential for fermenting wine, beer, and other alcoholic drinks.

Medical Use: Utilized in chemotherapy and other pharmaceutical applications, including penicillin and statins (lipid-lowering agents).

- One of the characteristics of fungal infections is that their course is indolent "subacute" meaning the patient's condition changes slowly over time. This is the opposite of viral and bacterial infections, which are typically acute and cause rapid changes in the patient's status

General mycology

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

1. Yeast:

- These are oval or round cells that reproduce by budding
- May form pseudohyphae (chains of elongated budding cells)
- *Candida albicans and Creptococcus neoformans*

Yeasts reproduce by budding, where the daughter cell is smaller than the parent cell.

Pseudohyphae are not tubular and lack true septation; instead, they show constrictions at their junctions, whereas hyphae have complete cross-walls.

Candida albicans and Cryptococcus neoformans

Candida: Certain Candida species are commensal organisms, forming part of the normal human flora, and infections caused by them are called candidiasis.

Cryptococcus neoformans: This species is consistently pathogenic and poses an occupational risk to people handling pigeons, leading to an exogenous, neurotrophic infection called cryptococcosis, a potentially serious fungal disease that often affects the CNS.

Note!
Pleomorphic means having more than one shape or form.
·Candida species {like Candida albicans} are called pleomorphic fungi because they can exist in different morphological forms, such as:
◦Yeast form→ single oval cells that reproduce by budding
o Pseudohyphae→ chains of elongated budding cells
◦True hyphae→ long filamentous structures

Two Types of Fungal Infections

1.Endogenous Infections

Caused by fungi that are part of the normal human flora.

Common sites: skin, upper respiratory tract, female genital tract (vagina).

Triggered by:

Change in anatomical location, alteration in fungal characteristics, use of antibiotics

Example: Candida albicans

2.Exogenous Infections

Caused by fungi from external sources, not normally part of the human flora.It's always pathogenic.

Example: Cryptococcus neoformans

General mycology / yeasts cont’d

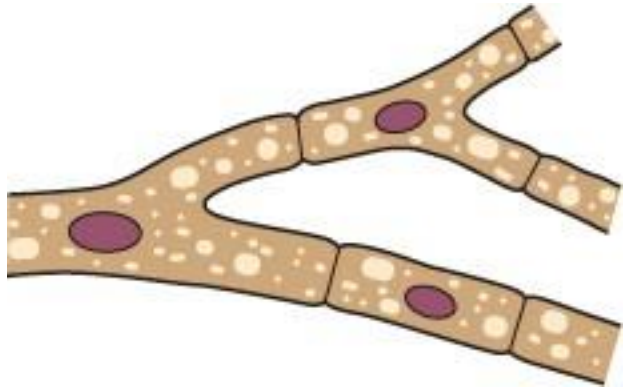
- Common in immunocompromised patients and can cause multisystem infections such as meningitis, arthritis and respiratory infections.

- *C. neoformans* found in soil and pigeon faeces and it commonly infects lung initially.

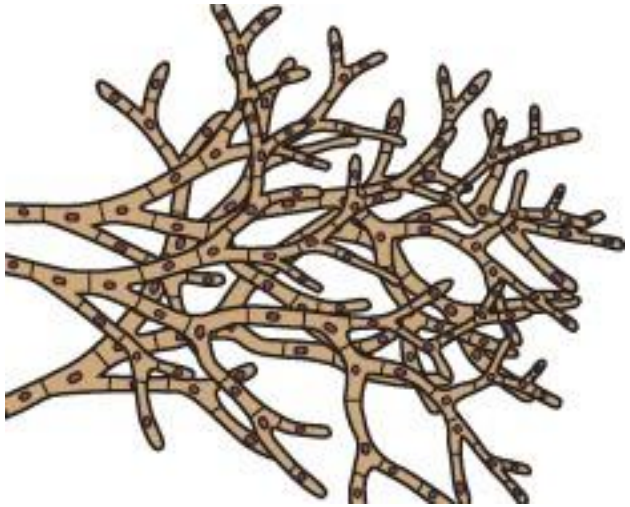
General mycology

2. Filamentous fungi (Molds):

- They have branching tubular filaments (hyphae) which may be septate or non- septate (Coenocytic hyphae)



- Mycelium: mass of branching, interlinking hyphae (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, Aspergillus and Dermatophytes



Zygomycetes (Mucorales),
Aspergillus (Aspergillosis)
dermatophytes (ring worms).

Some differences between yeast and hypha

Yeasts are unicellular fungi, so their thallus consists of a single cell, and they do not form a mycelium. In contrast, hyphae are multicellular filaments, and a network of hyphae forms the mycelium, which is part of the thalus, representing the entire fungal body.

Yeast reproduces by budding, where a small daughter cell forms and separates from the parent cell, showing a unicellular mode of growth. In contrast, hyphae reproduce through **elongation and branching** of their thread-like filaments, creating a multicellular network called mycelium.

- We called these extensions *Aerial extensions or aerial hyphae*

General mycology

3. Dimorphic fungi

- These occur in two forms: yeast form in tissues or when grow at 37˚C& filamentous form when grow at 22˚C

- Examples: Of endemic/ systemic mycotic diseases

Blastomyces dermatitidis Blastomycoses

Coccidioides immitis Coccidioidomycosis

Histoplasma capsulatum Histoplasmosis

Paracoccidioides brasiliensis

Sporothrix schencki

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

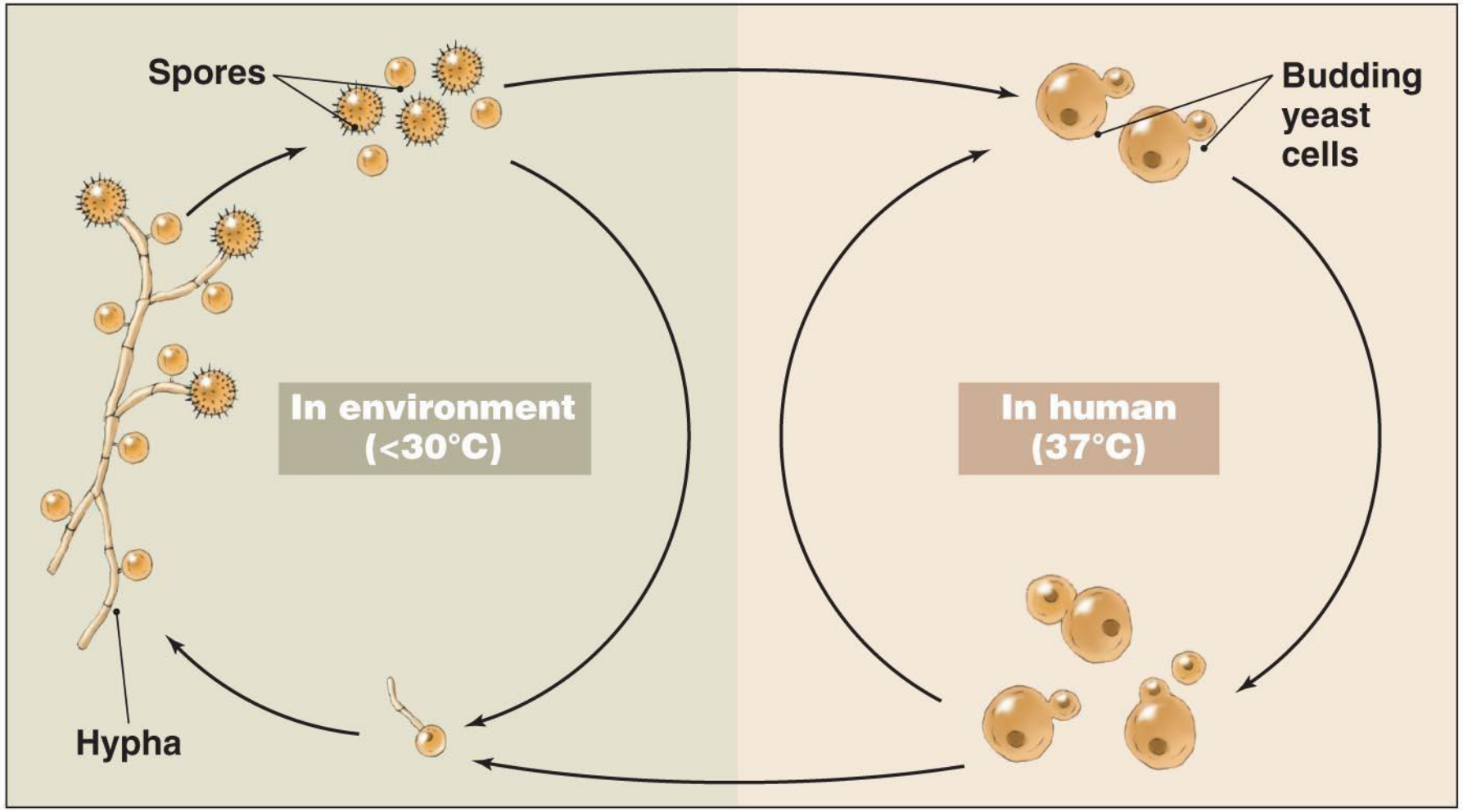


Figure 22.2

General mycology

- **Fungal diseases**
- Fungal infections have recently emerged as a growing threat to human health, especially to persons 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.

In fungal allergies, exposure occurs mainly through inhalation of spores, whereas in fungal intoxications, it happens through ingestion of toxins.

- 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

It can lead to external allergic rhinitis, with mild cases typically presenting as rhinorrhea, while more severe cases may progress to asthma.

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

2. Fungal toxins (mycotoxicosis):

- **Aflatoxicosis**

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

  - Aflatoxins are 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& resulting in production of aflatoxins.

Aflatoxicosis

- 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 induce mutation in the p53 human suppressor gene, leading to loss of growth control in hepatocytes.

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.

General mycology

There are 5 classifications of fungal infections: Superficial, cutaneous, subcutaneous, systemic and opportunistic fungal infections.

3. Fungal infection (mycoses):

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

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

The number has risen due to factors such as the use of immunosuppressants, broad-spectrum antibiotics, and conditions like diabetes, which also increase the risk of these infections.

General mycology

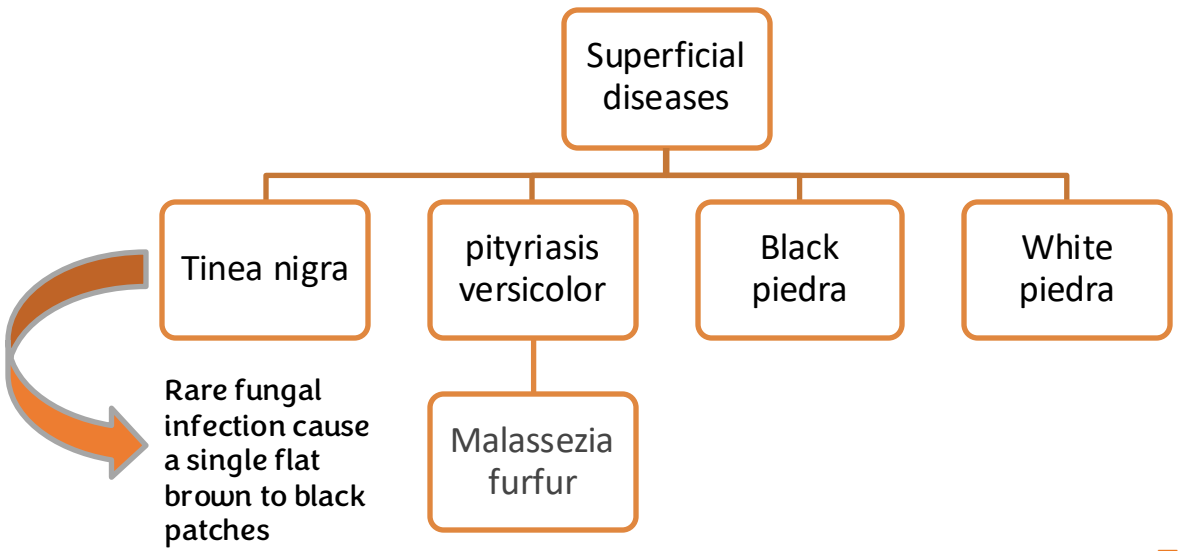
- ***Human fungal infections are commonly classified*** (accordingly to their anatomical and histological localization)***as:***

❖ **Superficial (**The outer most layer of the skin (stratum corneum) **)& Cutaneous infections**

- Infections involve the skin, mucous membrane, nail or hair with or without tissue destruction & immunological reaction
- e.g pityriasis versicolor , Tinea nigra
- e.g cutaneous candidiaisis (we will get to know about it later) & dermatophytes.

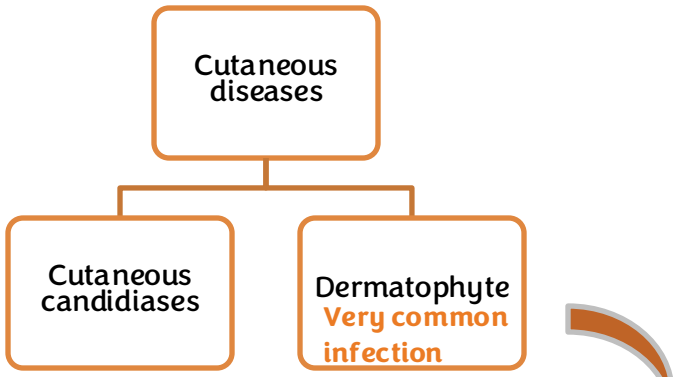
The superficial infection- there's no symptomatolgy or inflamatory response in the histopathological section of the outer most layer so the patient's visit to the doctor for cosmetic reasons after noticing the unusal patches of lighter (depigmentation) and darker skin (hyperpigmentation). **NO PAIN NO ITCHING**

But Cutaneous infections- there are pathological immunological and symptomatology



pityriasis versicolor caused by Malassezia furfur and it's has more prevalent

Will know Later on ... has a lot of clinical forms



Causes

Tinea (ring warm) and usually we add a word after "tinea" to describe the location of the body part where the infection is

- Tinea pedis: on the feet
- Tinea capitis on the scalp
- Tinea corporis on other part of the body

General mycology

❖**Subcutaneous** **It must had happened traumatic implantation so it can reach subcutaneous (fascia,cornea and muscle)**

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

  e.g Chromoblastomycosis

  There are 3 types of subcutaneous infection that can cause disease to humans

Chromoblastomycosis	**Sporotrichosis** **Rose Gardner disease**	**Madura foot**
Caused by different species but the most common - Fonsecea pedora - Phialophora verrucousa - CladoPhialophora Corrinii	Caused by - Sporathix schenkii (dimorphic fungus) 	Caused by - Madurella mycetomatis For the next lecture to now more about it

Small painful pumps appear on the skin along the lymphatic pathway vessels as the infection developed the lymphatic nodules because that gardeners who work with roses and thorns and get pricked by them the hyphea on the thorns converts to yeast in the body

General mycology

❖ Systemic mycoses

- Are primarily pulmonary lesion that may disseminate to any organ.
- **e.g COCCIDIOIDOMYCOSIS , HISTOPLASMOSIS**

The four main systemic dimorphic fungi :

- Histoplasma capsulatum (Causes Histoplasmosis)
- Blastomyces dermatitidis (Causes Blastomycosis)
- Paracoccidioides brasiliensis (Causes Paracoccidioidomycosis)
- Coccidioides immitis or Coccidioides posadasii (Causes Coccidioidomycosis, or Valley Fever)

They are specific to certain geographic areas and called endemic mycotic diseases (And luckily there's none in Jordan)

❖ Opportunistic Mycoses → Candida on the top of them as we have it normally

- ***e.g Candida* spp. *Cryptococcus***

→ Cryptococcus always pathological

Depends on :

- Species of fungus
- The host

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.

How to diagnose these infections? You need clinical suspicion (if the clinical picture shows the patient is not responding to an antibiotic) or…
The illness is not acute (subacute or chronic) يكون خلال ايام و اسابيع و ليس مفاجئ
… and if the patient has predisposing factors (like: Immuno compromised – low immunity) Diabetes
So they develop the infection

- **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

1. How to do laboratory diagnosis?
2. Direct Microscopy under the microscope.
3. Gross Appearance (by your eyes).
4. Culture and Isolation (the harder way) by isolation and culture like bacteria on artificial agar plate.
5. Serological tests look for antigen to this species in the patient's blood or an antibody for this antigen in the patient's blood.
6. Molecular (DNA) methods use DNA probes (PCR) to certain DNA from the genome of the fungal species: Histoplasma capsulatum and Cryptococcus neoformans.

Types of specimen

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

When collecting a specimen for superficial and cutaneous mycotic infections, we take skin scrapings from the active edge of the lesion. In pityriasis versicolor, the lesion is characterized by fine, scale-like patches, or furfuraceous scales, from which we can collect a specimen for laboratory analysis

- 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.

Necessity for Deep Sampling: Subcutaneous fungi (e.g., in Madura foot, Chromoblastomycosis) affect deeper tissues like the dermis or fascia, making superficial samples insufficient.Primary Deep Method: Aspirated pus is typically collected from the deep infection site using needle aspiration.Definitive Diagnosis: A tissue biopsy is often the gold standard, particularly when the fungi are deep-seated or for confirming the diagnosis.Surface Samples: Scrapings and crusts may still be collected from the overlying skin for initial screening or if the infection is tracking to the surface.

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

For suspected systemic fungal infections like Histoplasmosis or Blastomycosis, the sample collection site depends on the affected organ

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. Strong alkaline

The strong alkaline will dissolve all the cell walls of the bacteria and cell membrane so it'll only give us the fungal cells

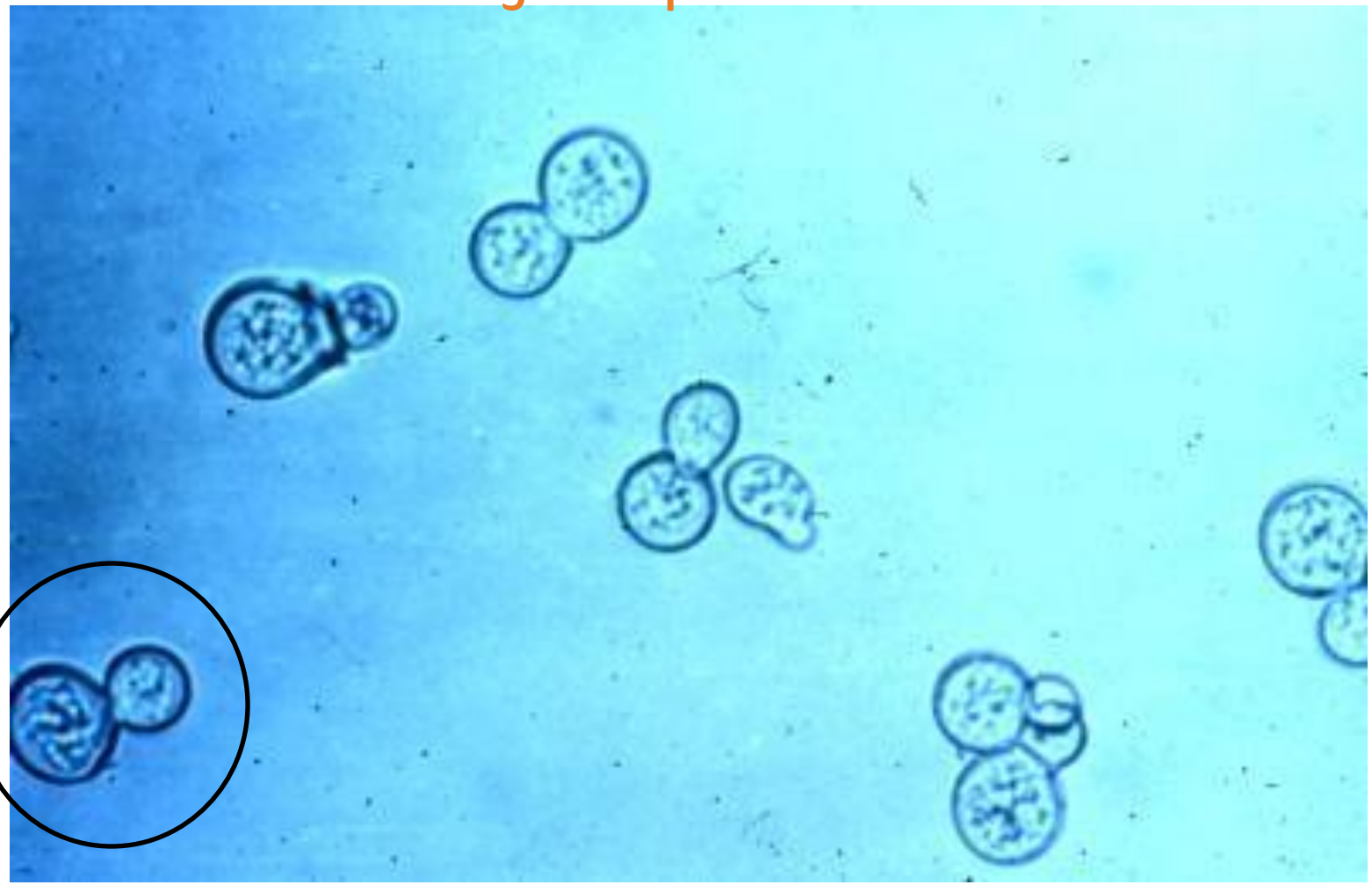
- Addition of *Calcofluor white* and subsequent examination by fluorescence microscopy enhances the detection of most fungi as the fluorescent hydroxide– Calcofluor binds to the fungal cell walls

Calcofluor white : binds directly to the cell wall of the fungi to see the fungi more clearly

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

KOH wet mount

After dissolving the specimen in KOH



Yeast

round and oval

Light microscope

Culture (for more complex cases we need to isolate to the specimen and transfer it to Agar plate that have bacteria can growth fungi)

- Most pathogenic fungi are easy to grow in culture.

contamination (the unintended presence of foreign substances, which can be chemicals ,physical matter, or microorganisms ,leading to inaccurate test results ,compromised samples, and potential waste of resources)

Universal agar

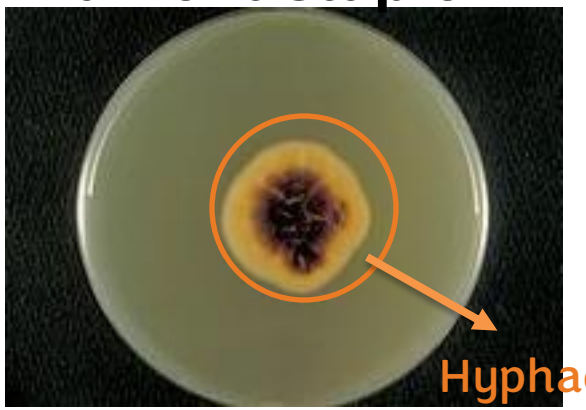
Sabouraud dextrose medium:

- commonly used
- may be supplemented with chloramphenicol to minimize bacterial contamination and cycloheximide to reduce contamination with saprophytic fungi. So it will only give us the pathogenic fungi

It's a problem that the agar face because of the other fungi in the room

Candida

filament stapler



(hairy/ wooly كومة شعر)

Antifungal therapy

Briefly they are only six classes so their amounts much lesser than antibiotics even in the hospital only 2 of them can be taken intravenously and the others orally and topically (shampoo ointment)

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

- it depends on presence of ergosterol in fungal cell membranes

- Amphotercin B and nystatin are polynes (Fungicidal) & various azoles (fungistatic) are commonly used for treatment of fungal infection.

Kill fungal cell
)administrated usually intravenously)

Inhabits multiplying and growing fungal cells

Depending on the seriousness of the infection We take fungicidal or fungistatic

General mycology

1. Polyene derivatives(fungicidal) → Mechanism: Binds directly to the ergosterol)similar to cholesterol but found in fungi) in the cell wall so this disrupt it and then kill the cell
   - Amphotericin B — Only by IV
   - Nystatin

   Can be used for out patient fungal infection –there's topical and oral
2. Azoles → azoles, bind to an intermediate step in ergosterol biosynthesis. By preventing lanosterol from converting into ergosterol, they stop the process.
   - Ketoconazole
   - Fluconazole
   - Itraconazole
   - Voriconazole
   - Posaconazole

Dandruff قشرة الشعرis kind of a fungal infection and we use mizoral shampoo which is a treatment

General mycology

3. Griseofulvin

Preventing mitotic spindles formation, binds to its target tubulin so this prevent multiplying fungal cells

4. 5-fluorocytosine (5-FC)

Analog for cancer patients in chemotherapy and it's very serious potent DNA & RNA inhibitor

5. Allylamines

Work on the earliest sltep of ergosterol on cell membrane of the fungal cell bind to squalene epoxidase

-Terbinafine (Lamasil)

Can be Ointment, shampoo

6. Echinocandins (fungicidal)

Work by inhibitory glucagon synthase they target the cell wall not cell membrane

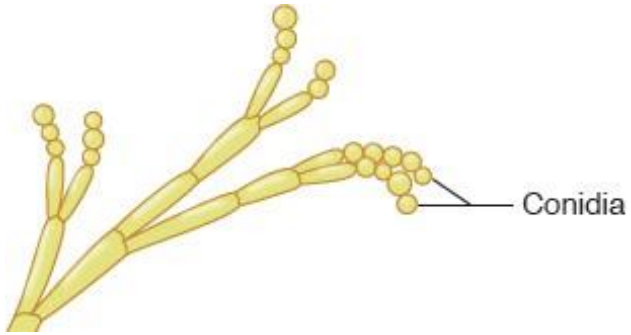
- Caspofungin

Only by IV

The End

Spore types

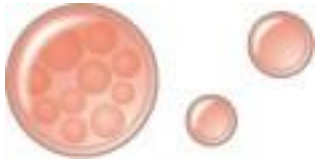
- Conidial Asexual spores l Formed off of hyphae l Common l Airborne



- 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



Additional Resources:

رسالة من الفريق العلمي:

"وقد أجمع عقلاء كل أمة أن النعيم لا يُدرك بالنعيم،وأن من آثر الراحة فاتته الراحة،وأنه بحسب ركوب الأهوال واحتمال المشاق تكون فرحة اللذة"

مفتاح دار السعادة: ابن القيم رحمه الله

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slid 29	the four main systemic dimorphic fungi 1. *Histoplasma* 2. *Capsulatum* 3. *Blastomyces* 4. *Paracoccidioidiosis*	the four main systemic dimorphic fungi : • **Histoplasma capsulatum** (Causes Histoplasmosis) • **Blastomyces dermatitidis** (Causes Blastomycosis) • **Paracoccidioides brasiliensis** (Causes Paracoccidioidomycosis) • **Coccidioides immitis** or **Coccidioides posadasii** (Causes Coccidioidomycosis, or Valley Fever)
V1 → V2			