



AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 12.

Microbiology diagnosis of mycoses

FACULTY: General Medicine

SUBJECT: Medical microbiology - 2

Discussed questions:

1. Classification of *fungi* that are pathogenic to humans.
2. Classification of mycoses: superficial mycoses (keratomycoses), skin mycoses (dermatomycoses), subcutaneous mycoses (subcutaneous) mycoses, systemic or visceral mycoses and opportunistic mycoses.
3. Morpho-biological features of the causative agents of *superficial mycoses* (tinea versicolor, black and white piedra), principles of microbiological diagnosis of the diseases they cause
4. Morpho-biological characteristics of the causative agents of *skin mycoses* (trichophytia, epidermophytia, microsporia, favus), principles of microbiological diagnosis of the diseases they cause
5. Morpho-biological characteristics of the causative agents of *subcutaneous* mycoses (sporotrichosis, chromomycosis, mycetoma), principles of microbiological diagnosis of the diseases they cause
6. Morpho-biological features of the causative agents of *systemic* (visceral) mycoses (coccidioidosis, histoplasmosis, blastomycosis, paracoccidioidosis), principles of microbiological diagnosis of the diseases they cause
7. Morpho-biological characteristics of the causative agents of *opportunistic* mycoses (candidiasis, cryptococcosis, aspergillosis, mucoromycosis, pneumocystosis), the principles of microbiological diagnosis of the diseases they cause
8. The concept of mycotoxicosis

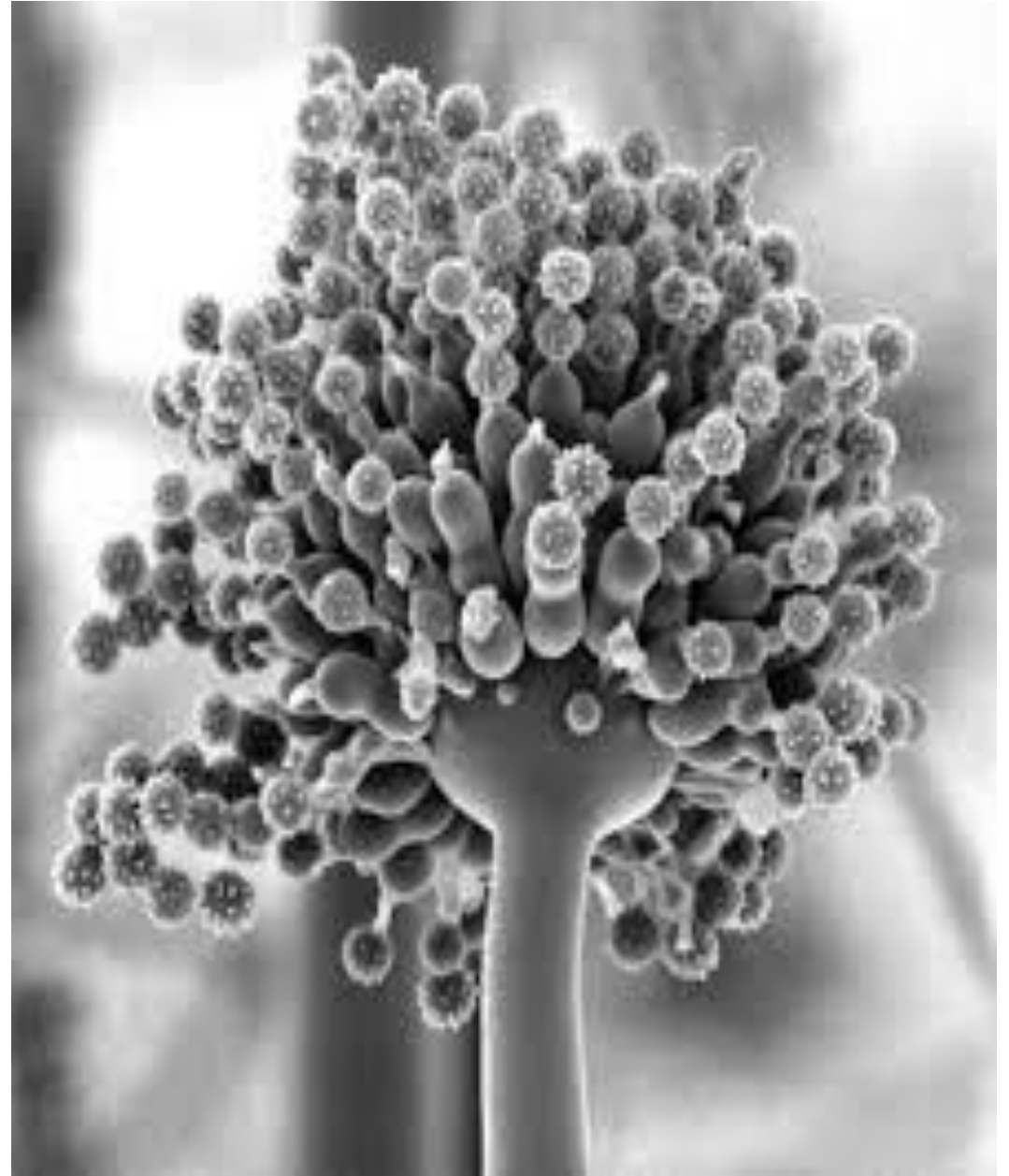
Purpose of the lesson:

- To acquaint students with classification of mycoses, morpho-biological characteristics of their causative agents, pathogenesis of mycoses, microbiological diagnosis, principles of treatment and prevention.

Fungi (Fungi, Mycetes, Mycota) are plant-based, chlorophyll-free, single- or multi-celled eukaryotic organisms

The branch of microbiology that studies fungi is called mycology

There are pathogenic and non-pathogenic types of fungi

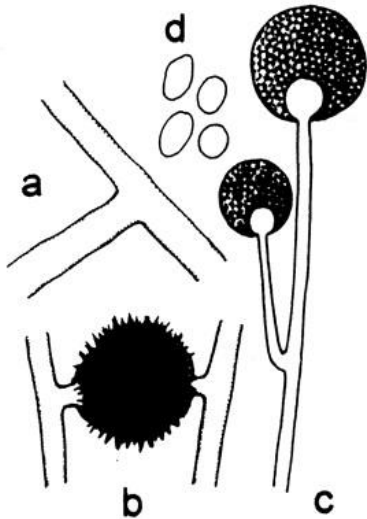


KINGDOM

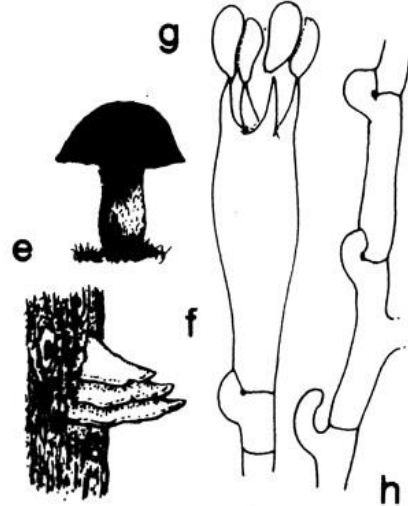
FUNGI

PHYLUM

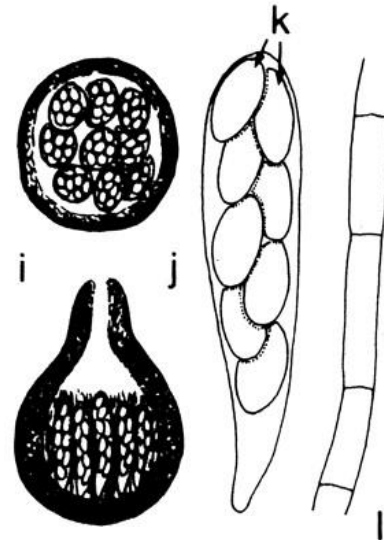
ZYGOMYCOTA



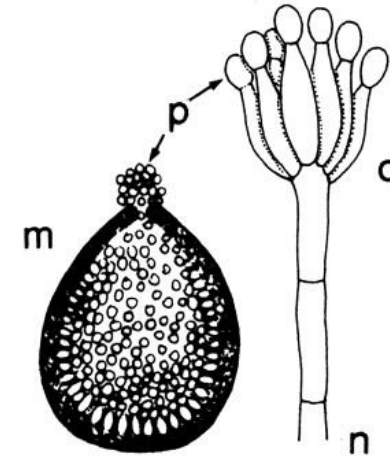
BASIDIOMYCOTA



ASCOMYCOTA



**DEUTEROMYCETES
(MITOSPORIC FUNGI)**



Classification of fungi that are pathogenic for humans:

Zygomycota	<i>Rhizopus</i> <i>Absidia</i> <i>Mucor spp.</i>
Ascomycota	<i>Blastomyces</i> <i>Histoplasma</i> <i>Microsporium</i> <i>Trichophyton</i> <i>Coccidioides</i> <i>Saccharomyces</i> <i>Candida spp.</i>
Basidiomycota	<i>Cryptococcus neoformans</i>
Deuteromycota	<i>Epidermophyton</i> <i>Paracoccidioides</i> <i>Sporothrix</i> <i>Aspergillus</i> <i>Phialophora</i> <i>Fonsecaea</i> <i>Exophiala</i> <i>Cladophialophora</i> <i>Bipolaris</i> <i>Exserohilum</i>



MYCOLOGY

CLASSIFICATION OF FUNGAL INFECTION

- Superficial
- Cutaneous
- Subcutaneous
- Systemic
- Opportunistic

Mycogenic allergy - allergic conditions and diseases caused by mushrooms or their allergens;

Mycotoxicoes are pathological processes caused by metabolic products of microscopic fungi.

SUPERFICIAL MYCOSES

- 1. Pityriasis (Tinea) versicolor (*Malassezia furfur*)
- 2. Tinea Nigra – *Hortaea/Exophiala werneckii*
- 3. White Piedia – *Trichosporon*
- 4. Black piedra – *Piedraia*

Superficial: Surface mycoses

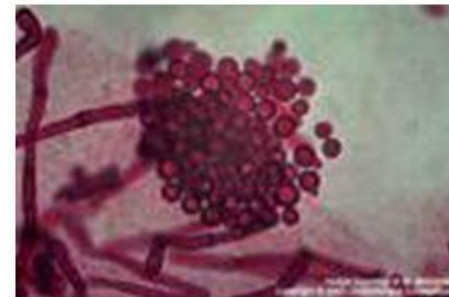
A. **Superficial mycoses** - infections limited to the outermost layers of the skin and hair. The superficial mycoses are:

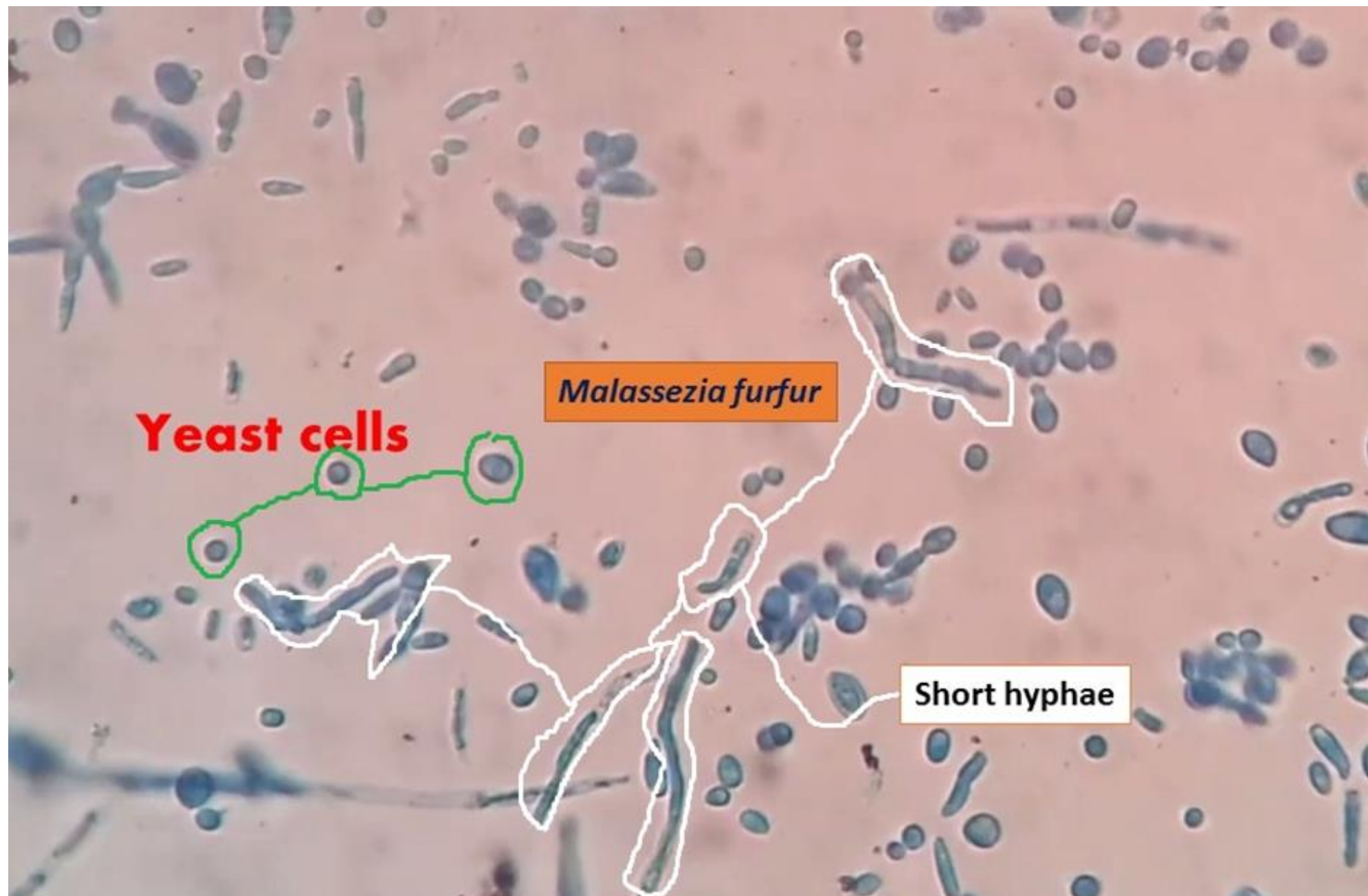
Disease	Etiological Agent	Symptoms	Identification of organism
Pityriasis versicolor	<i>Malassezia furfur</i>	hypopigmented macules	" <u>spaghetti and meatballs</u> " appearance of organism in skin scrapings
Tinea nigra	<i>Exophiala werneckii</i>	black macules	black, 2-celled oval yeast in skin scrapings
Black piedra	<i>Piedraia hortai</i>	black nodule on hair shaft	black nodule on hair shaft composed of spore sacs and spores
White piedra	<i>Trichosporum beigelii</i>	creme-colored nodules on hair shaft	white nodule on hair shaft composed of mycelia that fragment into arthrospores

Malassezia infections

Description:

- MALASSEZIA FURFUR (Lipophilic yeast) Saprophytes on skin's lipid secretions
- Malassezia furfur* is the causative agent of Pityriasis versicolor, Pityriasis folliculitis and it has recently been implicated as a causative agent of seborrhoeic dermatitis and dandruff.
- It has also been recovered in blood cultures from neonate and adult patients undergoing lipid replacement therapy.
- M. furfur* is a lipophilic yeast living on the skin as part of the normal flora.





Pityriasis (Tinea)Versicolor

Fungal infection of the skin

- ❖ This is a very common superficial mycoses caused by a lipophilic yeast *Malassezia* spp. It is seen in all countries but is particularly common in the tropics with more than 60 % of the population infected.
- ❖ The lesions are hypo or hyper pigmented. The etiologic agent is normal flora of man so the disease re-occurs.
- ❖ Multiple patchy lesions (oval shape c fine scales) either light in color or brown
- ❖ Typically occurs on the back, neck, chest, shoulders

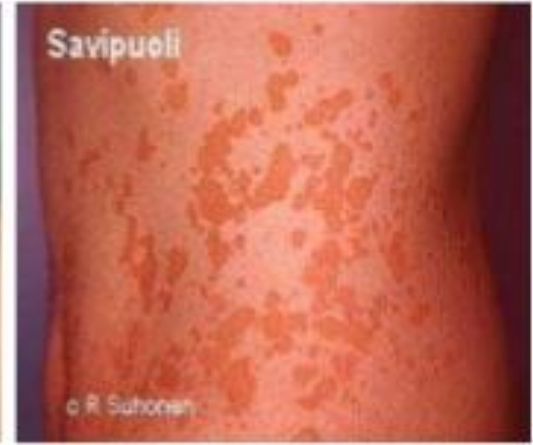
General Morphology

Tinea Versicolor



- **Numerous, well-marginated, oval-to-round macules** (change in the color of the skin) **with a fine white scale when scraped.**
- **Pigmentary alteration uniform in each individual.**
 - Red
 - Hypo pigmented
 - Hyperpigmented
- **Scattered over the trunk and neck. Seldom the face.**
- **M. furfur**
 - Normal flora of skin
- **Asymptomatic.**

Superficial mycosis (Pityriasis versicolor)



Superficial mycosis (Pityriasis versicolor)

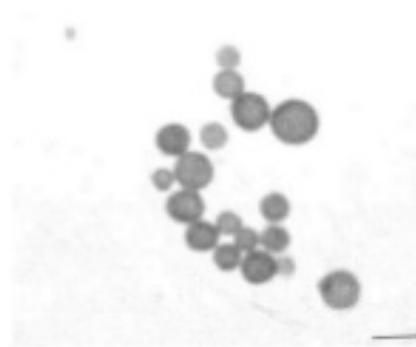


Tinea Versicolor

Malassezia furfur

Definition

- Asymptomatic colonization
- Hypopigmentation
- Hyperpigmentation
- Seborrhoeic dermatitis
- Dandruff
- Fungaemia



Pityriasis versicolor

- **Conditions:**
 - Health
 - Sweat
 - Greasy skin
 - Chronic bacterial infections
 - Steroids

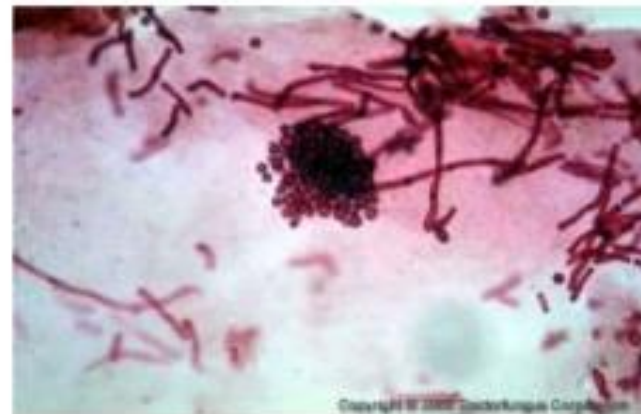
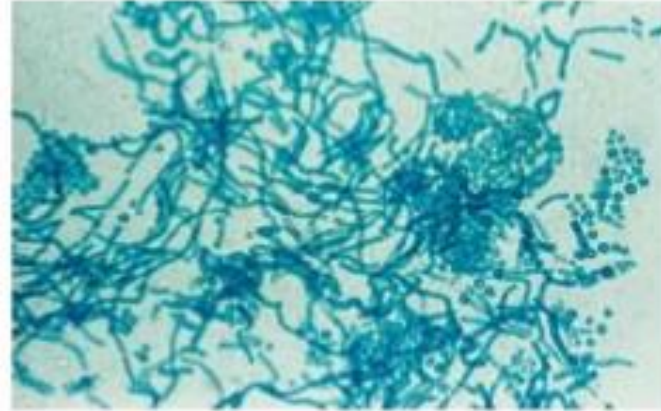
Pityriasis versicolor

- **Clinical manifestations:**
 - Maculae
 - White, cream, pink, red, brown
 - Scale/signs
 - Painless
 - Not itching

Pityriasis versicolor

Laboratory diagnosis:

- Sampling
 - Scalpel (sharp bladed instrument)
 - Wood's lamp (producing ultraviolet radiation)
- Direct
- Culture



Tinea Versicolor

Diagnosis:

- Scrape lightly – fine white scale
- KOH Positive for short hyphae and spores
(Spaghetti –hyphae and meatballs-yeasts)
- Woods Light – pale yellow white fluoresce.
- Culture rarely done.



Tinea Versicolor

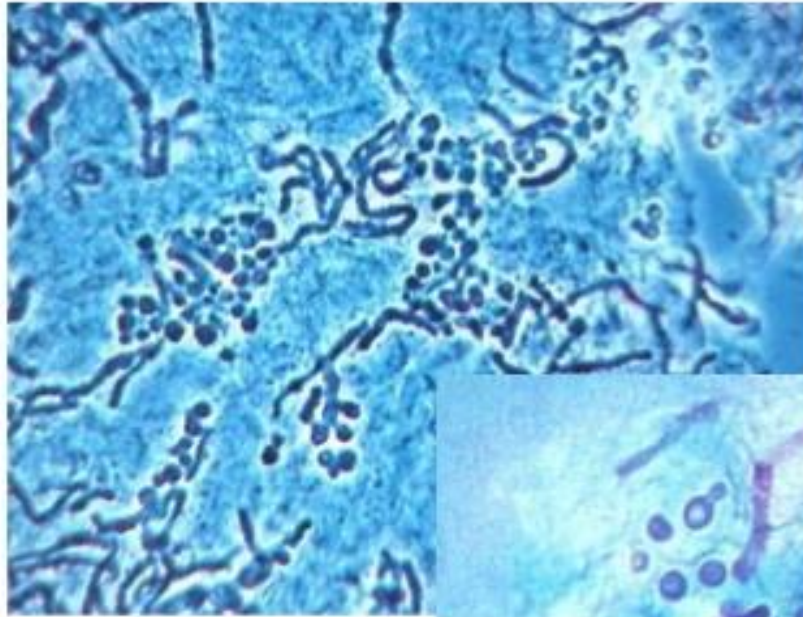
Direct exam.

- Skin scraping
- 10% KOH preparation
- Parker Ink

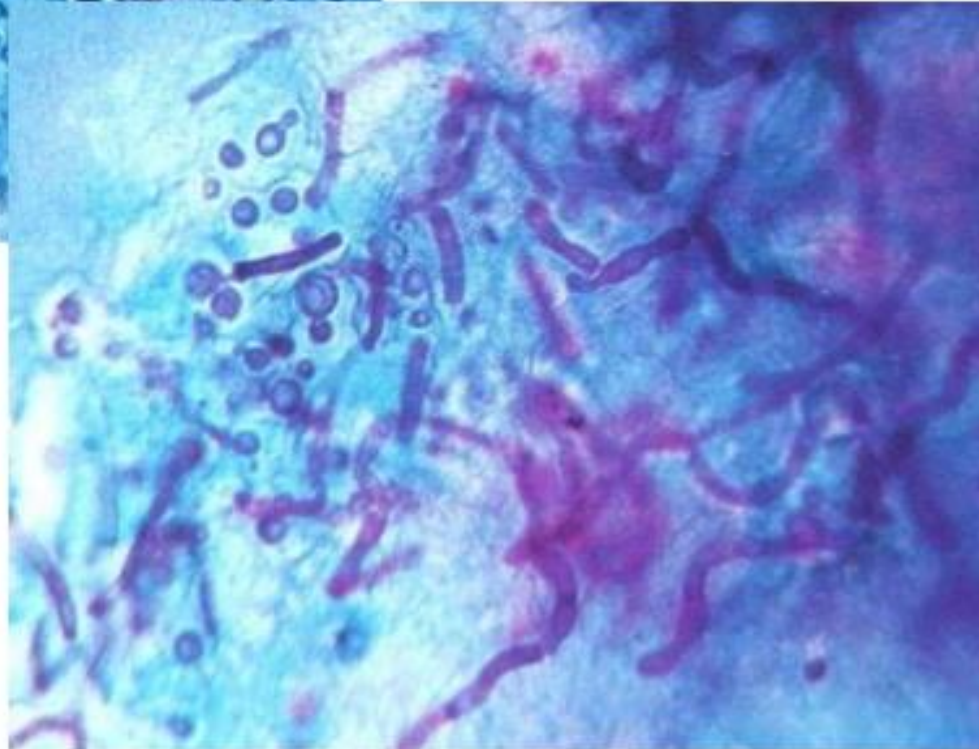
Culture

- Sabouraud Dextrose Agar
- Lipophilic yeast
- Flood with Olive oil

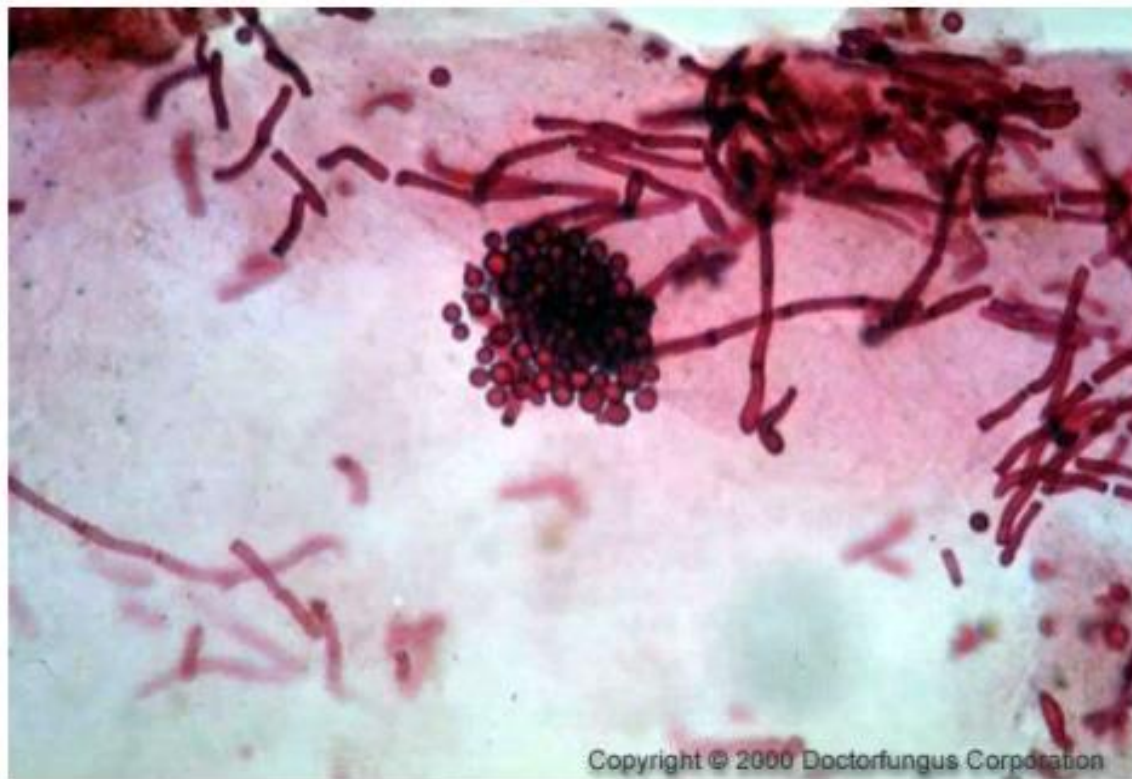




- Short fragments of hypha
- cluster of yeast



“Spaghetti and meatballs”



Culture

❖ *Malassezia furfur* can be grown in a laboratory but only when one covers the culture with oil. This leads me to speculate that there is a genetic susceptibility to this disease resulting from qualitative or quantitative differences in the oil content of susceptible persons.



	Black piedra	White piedra
Synonyms	Tinea nodosa, trichomycosis nodularis	Trichosporosis nodosa
Clinical features	Firmly adherent, black, gritty hard nodules	Soft, white or light brown, transparent, easily detachable nodules
Site of involvement	Mostly scalp Beard, moustache, pubic area: Less frequent	More commonly: Hairs of beard, moustache and genital areas Scalp: Less common
Causative organism	<i>Piedraia hortae</i>	Genus <i>Trichosporon</i> Scalp white piedra: <i>Trichosporon ovoides</i> Crural white piedra: <i>Trichosporon inkin</i> , <i>Trichosporon asahii</i> , <i>Trichosporon mucoides</i> Possible synergistic role of <i>Brevibacterium</i> (proteolytic activity)
Hair shaft breakage	Subcuticular fungal growth can cause rupture of cuticle and hair breakage occurs in longstanding cases	Growth of the fungus both within and outside the hair shaft may cause the hair shaft to weaken and break off
Geographical area	Mostly tropical areas	Mostly tropical areas, but found in temperate climates as well
KOH mount	Nodules composed of closely packed brown, dematiaceous hyphae with arthroconidia 4-8 µm in diameter at the edges of the nodule. The thick parts of the mature nodule show club shaped asci containing 8 elongated ascospores	Nondematiaceous, loosely arranged septate hyphae with arthroconidia (2-4 µm) and budding blastoconidia
Culture characteristics	Slow growth of compact, domed black colonies	Rapid growth of soft, creamy and wrinkly, sometimes mucoid colonies
Microscopic examination of culture isolate	Brown, thick walled, septate hyphae and chlamydoconidia Asci and ascospores in thicker portion of the colony	Hyphae, arthroconidia, budding cells. Physiological tests and genetic analysis required to differentiate the species

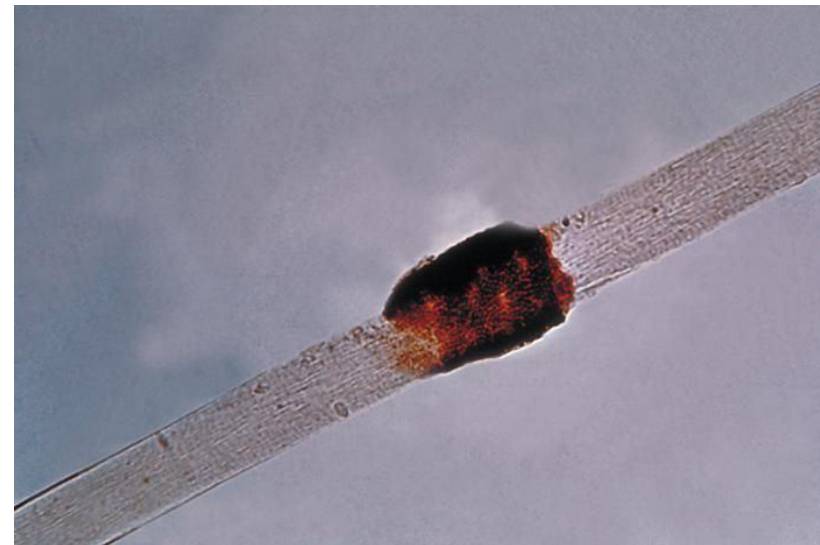
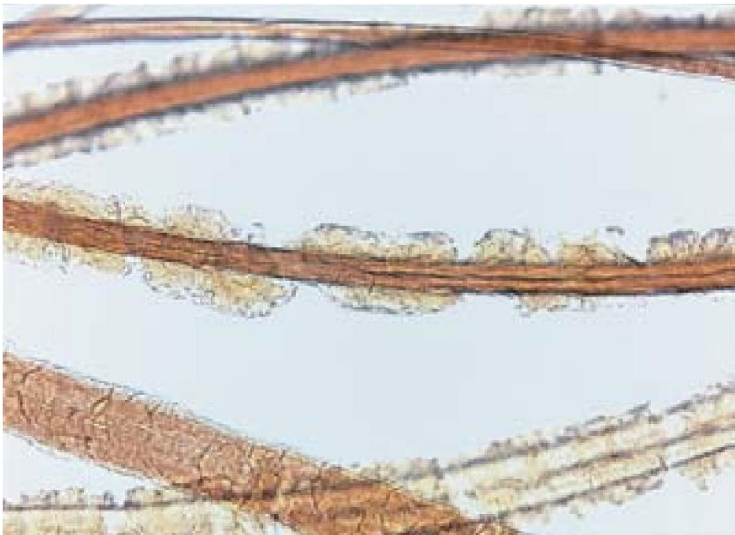
White piedra

Trichosporon asahii



Black piedra

Piedraia hortae



Cutaneous Mycoses



o**Dermatophytosis** - "ringworm" disease of the nails, hair, and/or stratum corneum of the skin caused by fungi called dermatophytes.

o**Dermatomycosis** - more general name for any skin disease caused by a fungus.

Cutaneous mycoses

- Infection of the skin, hair or nails caused by a group of keratinophilic fungi, called dermatophytes
- **DERMATOPHYTOSIS (=Tinea = Ringworm)**

DERMATOPHYTES

- Digest keratin by their **keratinases**
- Resistant to **cycloheximide**
- Classified into **three groups** depending on their usual habitat

Cutaneous mycoses

- Keratophilic – use keratin as subject to live (parasites)
- Keratinases- invade only keratinized layers

DERMATOPHYTOSIS

Classification

Three Groups/Types:


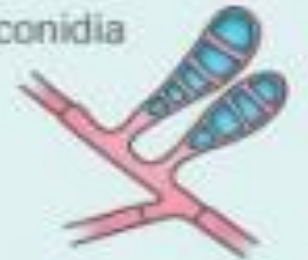








- Clinical
- Etiological
- Ecological

Etiology of Dermatophytes

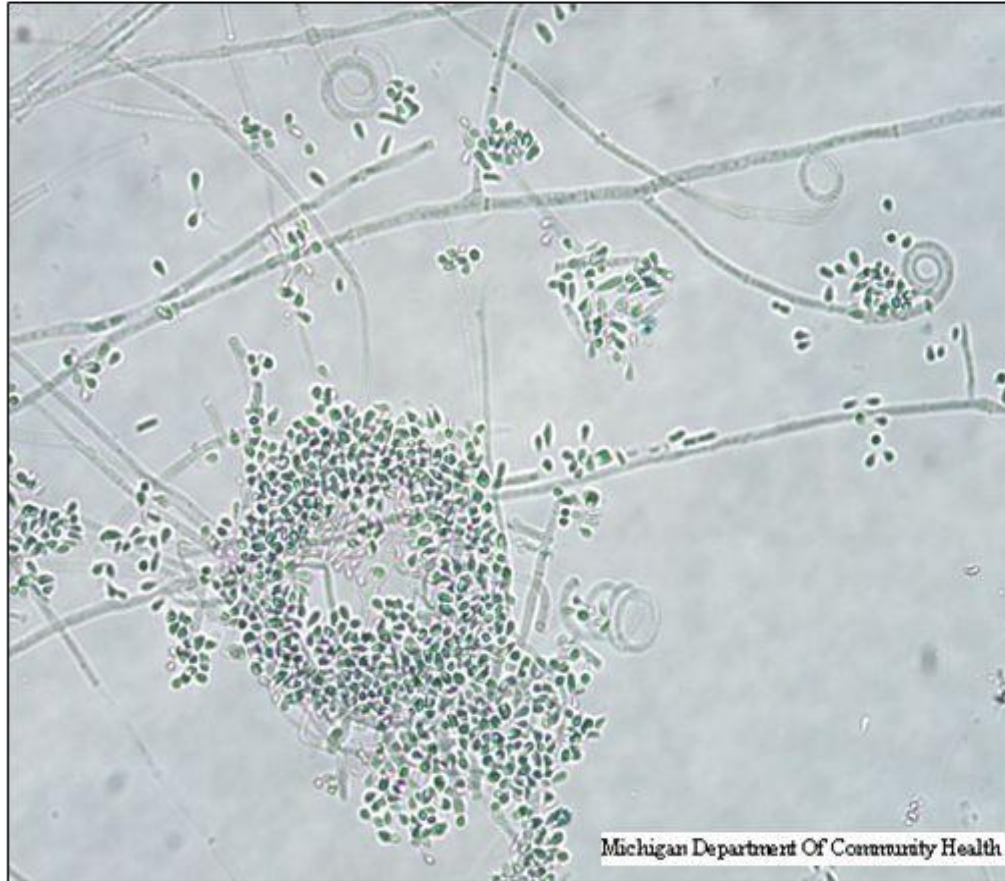
Etiology (3 Genera)

- Trichophyton
- Microsporum
- Epidermophyton

Features	Trichophyton	Microsporum	Epidermophyton
Site of infection	Skin, hair, nail	Skin, hair	Skin, nail
Macroconidia	Sparse, thin-walled, smooth, Pencil shaped	Numerous, thick-walled, rough, Spindle shaped	Numerous, smooth-walled, Club shaped
Microconidia	Abundant	Rare	Absent
Examples	T.rubrum, T.schoenleinii, T.violaceum, T.concentricum, T.mentagrophytes, T.tonsurans, T.equinum	M.canis, M.fulvum, M.nanum, M.andouinii, M.gypseum, M.racemosum, M.equinum, M.gallinae	E.floccosum

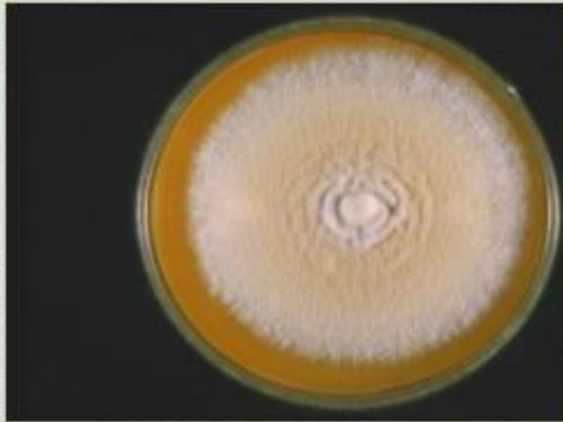
Conidia	Microconidia (<i>Microsporum</i> and <i>Trichophyton</i> only)	
	Macroconidia	  
	Chlamydoconidia	
	Terminal	
	Intercalary	
Hyphae	Arthroconidia	
		  

Trichophyton mentagrophytes

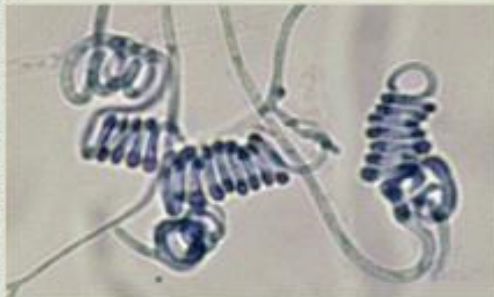


- ❖ Colony growth is moderately rapid, powdery to granular, white to cream colored on the surface with a yellowish, brown or red-brown reverse.
- ❖ Microconidia are numerous, unicellular, round to pyriform and found in grape like clusters. Spiral hyphae are often present.
- ❖ Macroconidia are multiseptate, club-shaped and often absent.
- ❖ Lab tests: hair perforation test positive, urease positive, growth at 37°C.
- ❖ Infection is typically found on the feet, hands, or groin, but can also be associated with inflammatory lesions of the scalp, nails, and beard.

Trichophyton mentagrophytes



Courtesy of
The Geraldine Kaminski Medical Mycology Library
Produced by: David Ellis and Roland Hermanis
Copyright © 2003 Doctorfungus Corporation



all types of tinea = spiral hyphae "en grappe"

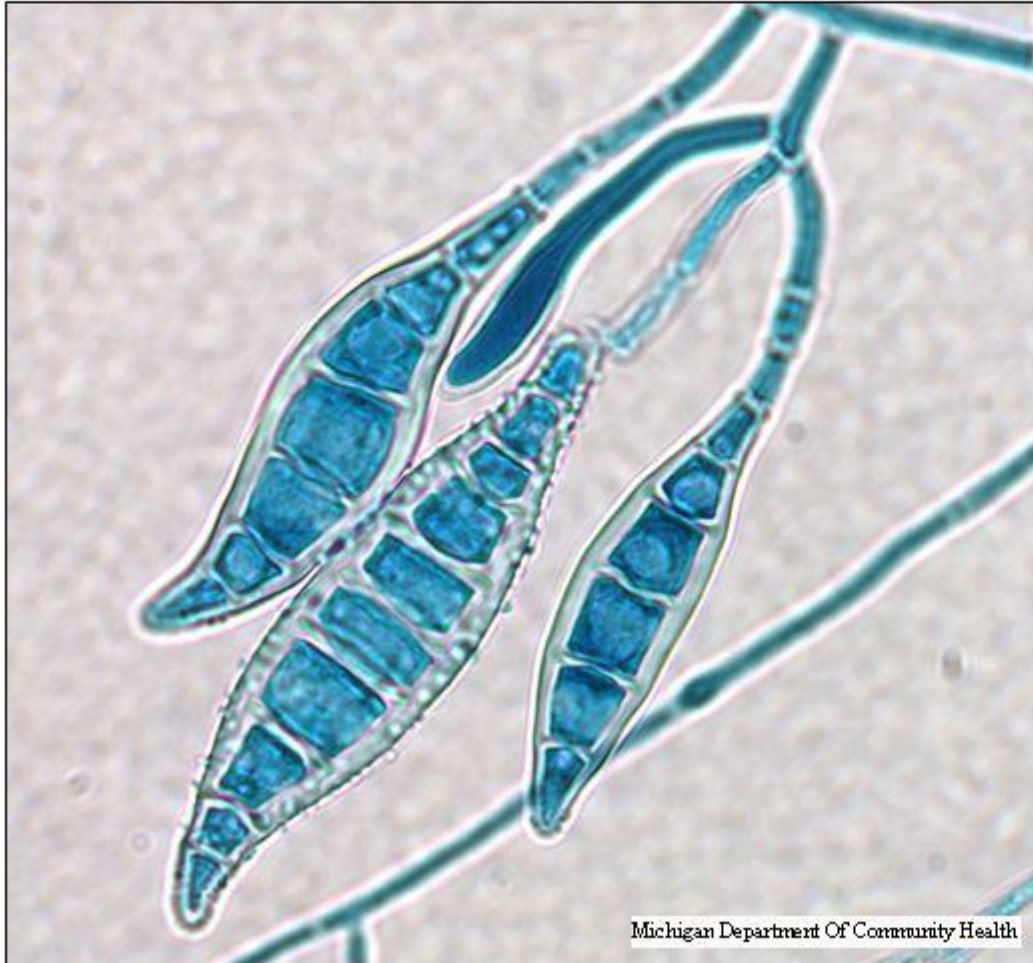
Trichophyton rubrum



Michigan Department Of Community Health

- ❖ Colony growth is slow to moderate, downy, white on the surface with a red to brown reverse.
- ❖ Microconidia are club-shaped to pyriform and are formed along the sides of the hyphae.
- ❖ Macroconidia are pencil-shaped to cigar-shaped.
- ❖ Lab tests: hair perforation test negative, urease negative, growth at 37°C.
- ❖ Infection is typically found on the feet, hands, nails, or groin.

Microsporum canis



- ❖ Colony growth is rapid, downy to wooly, cream to yellow on the surface with a yellow to yellow-orange reverse.
- ❖ Microconidia are club-shaped but typically are absent.
- ❖ Macroconidia are fusoid, verrucose, and thick walled. They have a recurved apex and contain 5-15 cells.
- ❖ Lab tests: hair perforation test positive and urease positive.
- ❖ Infection in humans occurs on the scalp and glabrous skin. It is also a cause of ringworm in cats and dogs.

Microsporum gypseum



❖ Colony growth is rapid, downy, becoming powdery to granular, cream, tawny-buff, or pale cinnamon on the surface with a beige to red-brown reverse.

❖ Microconidia are moderately abundant and club-shaped.

❖ Macroconidia are abundant, ellipsoidal to fusiform, sometimes verrucose, and thin walled. They typically contain 3-6 cells.

❖ Lab tests: hair perforation test positive and urease positive.

❖ Infection in humans is found on the scalp and glabrous skin; it is more frequently isolated from the soil and from the fur of small rodents.

Microsporium gypseum



Epidermophyton floccosum



❖ Colony growth is slow, powdery, with a yellow to khaki surface color and chamois to brown reverse.

❖ Macroconidia are club shaped, with thin smooth walls and can be solitary or grouped in clusters. Chlamydospores are often produced in large numbers.

❖ Microconidia are absent.

❖ Lab tests: hair perforation test negative, urease positive, growth at 37°C.

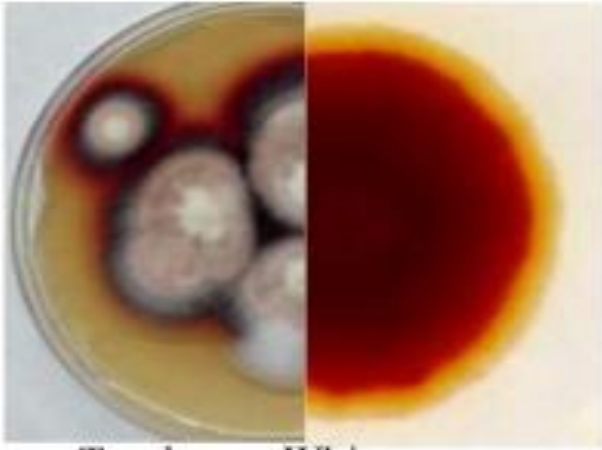
❖ Infections are commonly cutaneous, especially of the groin or feet.



*Epidermophyton
floccosum*

- Only one pathogenic species in this genus.
- **Tinea unguium** and **tinea cruris** are often caused by this fungus.





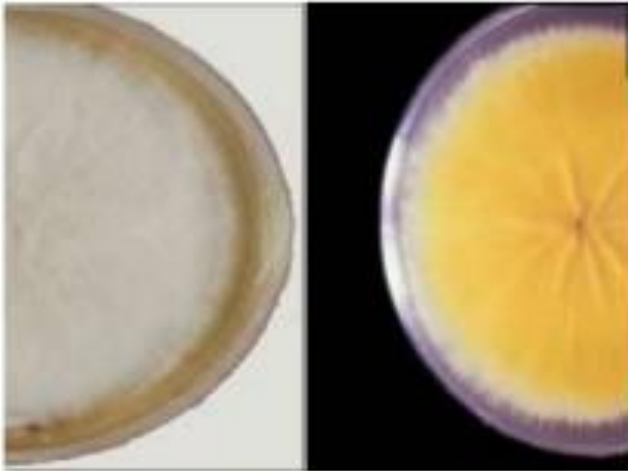
T. rubrum : White cottony surface and a deep red nondiffusible pigment from reverse side



T. tonsurans : flat, powdery, velvety colony.



T. metagrophytes : cottony to granular colony



Microsporum : white cottony surface with deep yellow from reverse.



Epidermophyton : flat, velvety with a tan to olive green tinge.



Dermatophyte test media

- **Diseases:**

- **Hairy areas:**

- Tinea capitis

Tinea barbae

- **Skin:**

- Tinea corporis
 - Tinea manum
 - Tinea fascie

Tinea cruris

Tinea pedis

Tinea imbricata

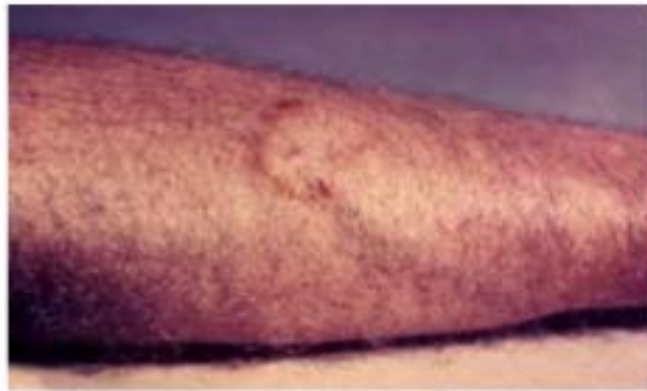
- **Nail:**

- Tinea unguium

Clinical Dermatophytosis

Clinical Classification & Manifestations

- Infection is named according to the anatomic location involved:
 - **Tinea corporis:**
small lesions occurring anywhere on the body



- **Tinea pedis :**

"athlete's foot". Infection of toe webs and soles of feet.



- **Tinea unguium (onychomycosis) :**
nails. Clipped and used for culture



- **Tinea capitis :**
head. Frequently found in children



Tinea barbae:

ringworm of the bearded areas of the face and neck.



Ecology of Dermatophytes

To determine the source of infection

- Anthropophilic
- Zoophilic
- Geophilic

Anthropophilic

- Associated with humans only. Person -to-person transmission through contaminated objects (comb, hat, etc.)
- e.g., *M. audouinii*, *T. tonsurans*

Zoophilic

- Associated with animals. Direct transmission to humans by close contact with animals.
- e.g., *M. canis*, *T. verrucosum*

Geophilic

- Usually found in soil (soil saprophytes). Transmitted to humans by direct exposure.
- e.g., *M. gypseum*, *T. ajelloi*.

DERMATOPHYTOSIS

Diagnosis

I. Clinical

Appearance

Wood's lamp (UV, 365 nm)

II. Lab

A. Direct microscopic examination

(10-25% KOH)

DERMATOPHYTOSIS

Diagnosis

B. Culture

- Mycobiotic agar
- Sabouraud dextrose agar
- Selective media – containing cycloheximide and chloramphenicol → incubate at 25 C.
- Identification based on the conidia

SUBCUTANEOUS MYCOSES

- Main
 - Lymphocutaneous sporotrichosis
 - Chromoblastomycosis
 - Eumycotic mycetoma
 - Subcutaneous zygomycosis
 - Subcutaneous phaeohyphomycosis
- Additional
 - Lobomycosis
 - Rhinosporidiosis

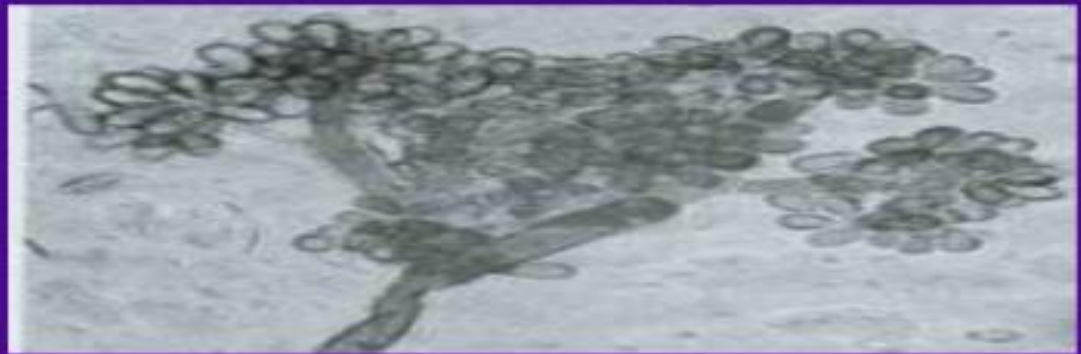
SUBCUTANEOUS MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
1. LYMPHOCUTANEOUS SPOROTRICHOSIS	<i>Sporothrix schenckii</i> – <i>thermophilic dimorphic Mold</i> – narrow, hyaline, septate – <i>condiophores</i> <i>Yeast</i> – spherical, oval (or) elongated	<ul style="list-style-type: none"> ➤ Skin by truma ➤ Small nodule – ulcerate ➤ Then discharge pus 	<ul style="list-style-type: none"> ➤ Culture of pus / tissue ➤ Immunologically – exoantigen test 	<ul style="list-style-type: none"> ➤ Oral potassium iodide ➤ Itraconazole, terbinafine /potassium iodide ➤ Fluconazole ➤ Local application of heat



SUBCUTANEOUS MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
2. CHROMOBLASTOMYCOSIS (Chromomycosis)	<p><i>Pigmented septated (dematiaceous) fungi</i></p> <p><i>Fonsecaea, Cladosporium, Exophiala, Cladophialophora Rhinocladiella Phialophora</i></p> <p><i>Form muriform cells (Sclerotic bodies, Medlar bodies)</i></p>	<p>➤ Legs & arms/shoulders, neck, trunk, face, ears.</p> <p>➤ Chronic, pruritic, progressive, resistant to treat.</p> <p>➤ Verrucous lesions to flat plaques</p> <p>➤ Cauliflower like growth</p>	<p>➤ Histopathologic findings of chestnut brown, muriform cells.</p>	<p>➤ antifungal therapy Itraconazole & terbinafine</p> <p>➤ Posaconazole with flucytosine</p>



SUBCUTANEOUS MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
3. EUMYCOTIC MYCETOMA	<i>Phaeoacremonium</i> <i>Curvularia</i> , <i>Fusarium</i> <i>Madurella</i> , <i>Exophiala</i> , <i>Pyrenochaeta</i> , <i>Leptosphaeria</i> <i>Scedosporium spp.</i> <i>Septate</i> <i>Chlamydoconidia</i>	➤ Foot & hand/back shoulders & chest wall also. ➤ Small, painless, subcutaneous nodule/plague - increase slowly & enlarge - disfigured	➤ Grains can be mounted ➤ Microscopic	➤ Amphotericin B, Ketoconazole, itraconazole ➤ Antifungal Therapy



Madura foot



MYCETOMA- causative agents

FUNGI

Madurella mycetomatis

Madurella grisea

Leptosphaeria senegalensis

Neotestudina rosatii

Fusarium moniliforme

Fusarium solani

ACTINOMYCETES

Actinomadura madurae

Actinomadura pelletieri

Nocardia brasiliensis

Nocardia asteroides

Streptomyces somaliensis



SYSTEMIC MYCOSES

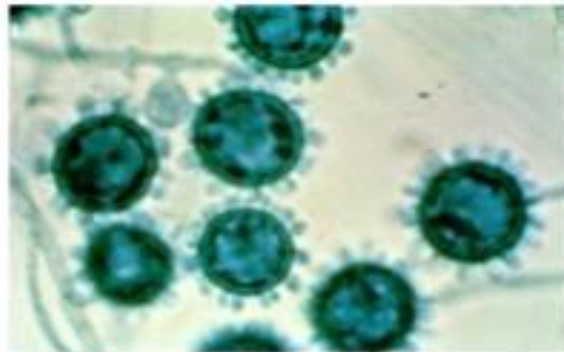
TRUE PATHOGENIC FUNGI

Genus	Species	Clinical conditions
Histoplasma	Histoplasma capsulatum	Histoplasmosis
Coccidioides	Coccidioides immitis	coccidioidomycosis
Blastomyces	Blastomyces dermatitidis	blastomycosis
Paracoccidioides	Paracoccidioides brasiliensis	paracoccidioidomycosis

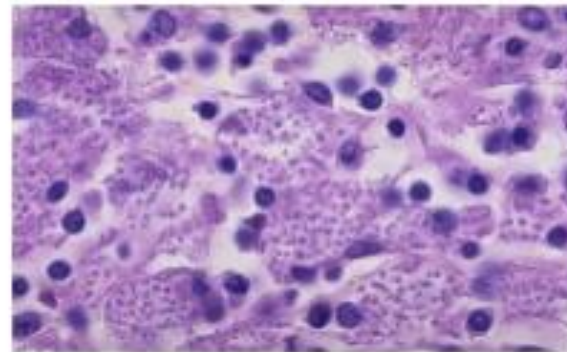
What does Dimorphic mean?

- Two forms exist for one fungus species depending on temperature and conditions of environment
 - Mycelial form - Hyphae and conidia
 - free living form found in nature and at laboratory temperature $\leq 30^{\circ}\text{C}$
 - Yeast or yeast like form
 - parasitic phase found in human tissue or in the lab $\geq 35^{\circ}$

Histoplasma capsulatum – mold
from 30°C culture



Histoplasma capsulatum – yeast
from tissue and 35°C culture



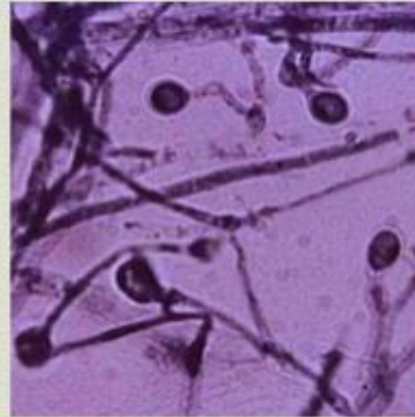
SYSTEMIC MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
1. BLASTOMYCES DERMATITIDIS	<p>BLASTOMYCOSIS</p> <p>Molds in culture - Branched septate Yeast like in tissue. – single budding yeast like</p> <p><i>Older culture may produce chlamydospores</i></p>	<p>➤ Present as pulmonary disease (or) extra pulmonary disseminated disease</p> <p>➤ Asymptomatic /present as a mild flu like illness.</p>	<p>➤ Suptum, pus, exudates, Urine & biopsy from lesions.</p> <p>➤ Microscopic examination</p> <p>➤ Culture</p> <p>➤ Serilogic test</p>	<p>➤ Itraconazole</p> <p>➤ Ketoconazole</p> <p>➤ Serious cases amphotericin B.</p>



Blastomyces dermatitidis

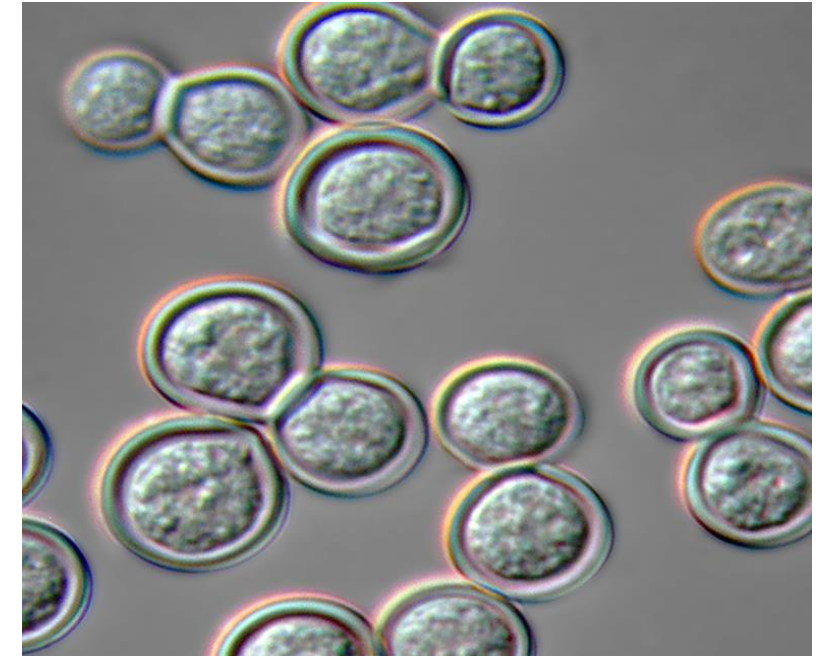
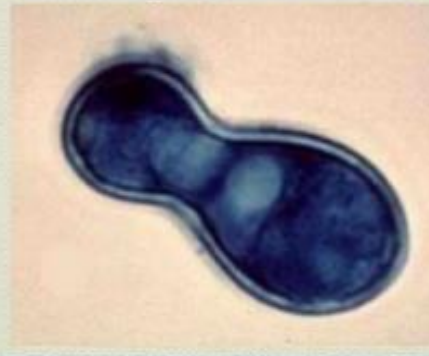
◆ MYCELIAL FORM

- ◆ The mycelial phase at 25° C showed typical pyriform microconidia , which are about 2-4 microns in diameter.



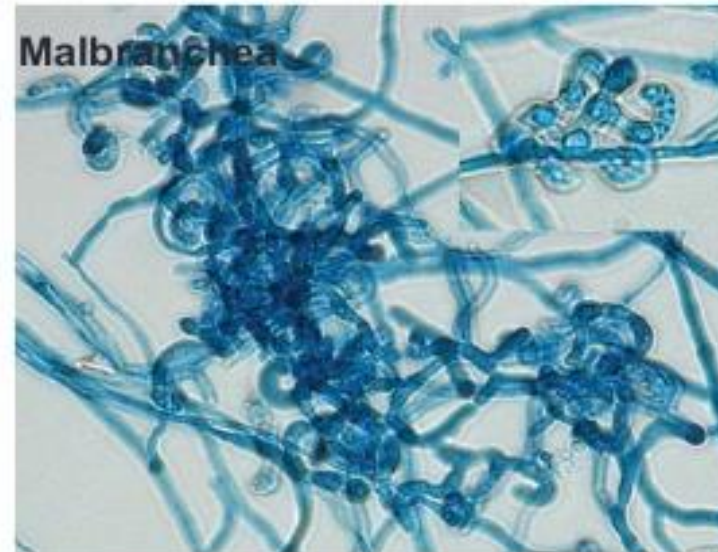
◆ YEAST FORM

- ◆ At 37° C and at body temperature, this organism is a yeast 8-15 microns in diameter.
- ◆ Buds are produced singly and are attached to parent cell by broad base.

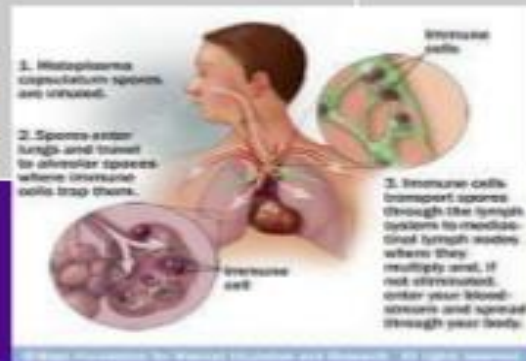
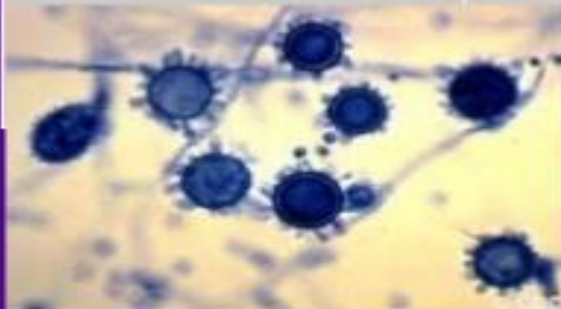


Coccidioides

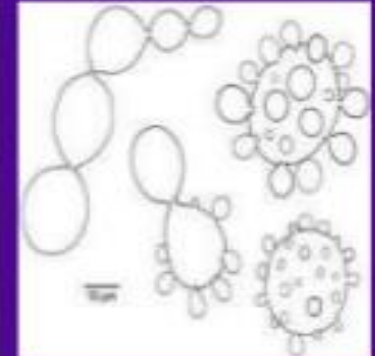
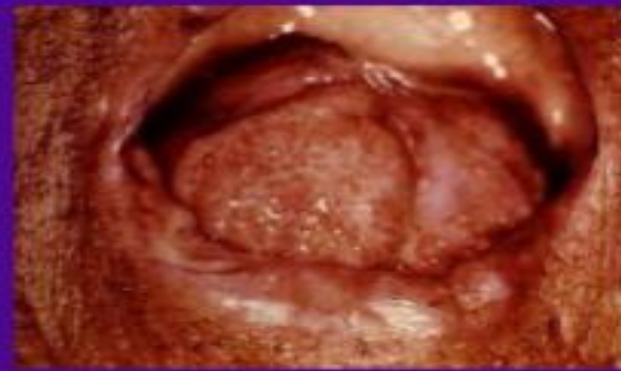
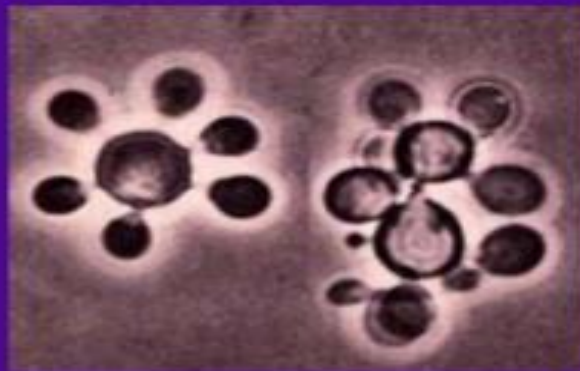
- Malbranchea species can look like C. immitis under the microscope
- Because of look-a-like fungi one needs to confirm identification of Coccidioides immitis with DNA probe or similar method to be sure!



SYSTEMIC MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
3.HISTOPLASMOSIS	<p>Histocapsulatum var Capsulatum, H. capsulatum var duboisii</p> <p>Molds in culture - hyaline septate - conidiophore</p> <p>Yeast like in tissue. – intracelluar budding yeast like</p>	<p>➤ Conidia develops into yeast cells – engulfed by alvelor macrophages</p> <p>➤ Initial inflammatory reaction because granulomatous</p> <p>➤ Mediastinal fibrosis</p>	<p>➤ Suptum, scrapings from superficial lesions, bone marrow, aspirates & buffy coat blood cells, blood films & biopsy from lessions.</p> <p>➤ Microscopic examination</p> <p>➤ Culture</p> <p>➤ Serilogic test</p>	<p>➤ Itraconazole</p> <p>➤ In disseminated disease, treatment with amphotericin B.</p>



SYSTEMIC MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
4.PARACOCCIDIOID OMYCOSIS	<p>Paracoccidioides brasiliensis</p> <p>Molds in culture - Branched septate</p> <p>Yeast like in tissue. – multiply budding yeast like</p> <p><i>Older culture may produce chlamydospores and conidia</i></p>	<p>➤ Present as pulmonary disease (or) extra pulmonary disseminated disease.</p>	<p>➤ Suptum, exudates & biopsy from lesions.</p> <p>➤ Microscopic examination</p> <p>➤ Culture</p> <p>➤ Serilogic test</p>	<p>➤ Itraconazole</p> <p>➤ Ketoconazole & Trimethoprim-sulfamethoxazole</p> <p>➤ Serious cases amphotericin B.</p>

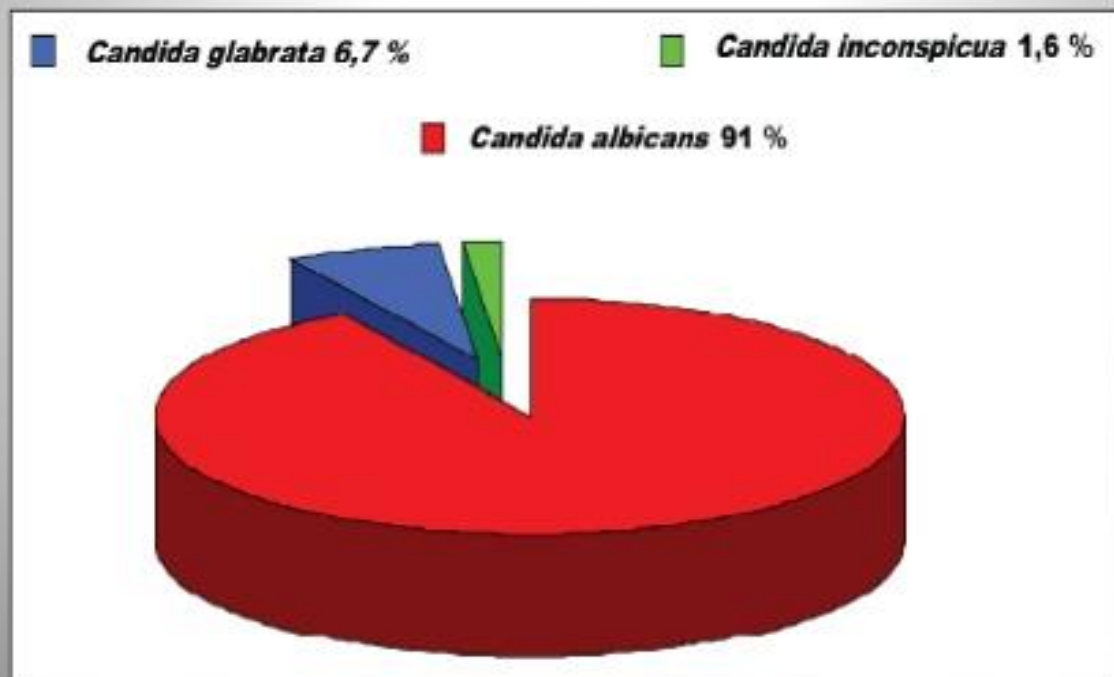


Opportunistic mycoses

Causative agents

- *Candida* species
 - *Cryptococcus neoformans*
 - *Aspergillus* species
 - *Zygomycosis* (*Rhizopus*, *mucor*, *absidia*)
 - *Penicillium* species
 - *Fusarium* species
 - *Alternaria* species
- ***ANY fungus found in nature may give rise to opportunistic mycoses ***

CANDIDIASIS



CANDIDIASIS

- *Candida albicans* is an opportunistic fungal pathogen that is responsible for candidiasis in human hosts.
- *C. albicans* grow in several different morphological forms, ranging from unicellular budding yeast to true hyphae with parallel-side wall .
- *Candida albicans* is a unicellular, oval-shaped diploid fungus (a form of yeast)
- Typically, *C. albicans* live as harmless commensals in the gastrointestinal and genitourinary tract and are found in over 70% of the population. Overgrowth of these organisms, however, will lead to disease

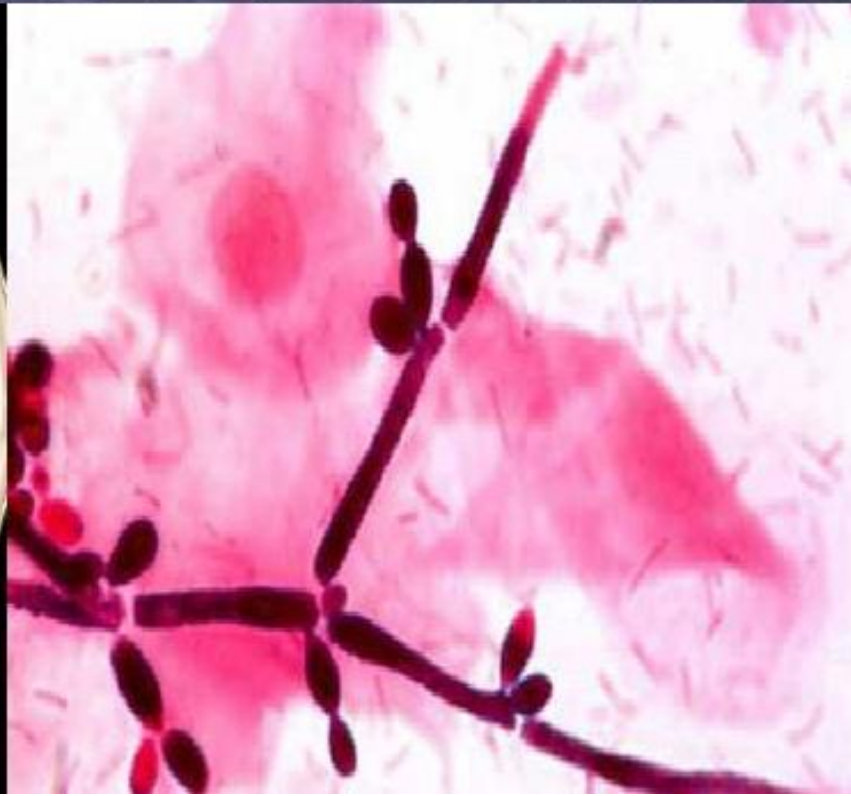
Morphology and Culturing

- Ovoid shape or spherical budding cells and produces pseudo mycelium
- Routine cultures are done on Sabouraud's Glucose agar,
- Grow predominantly in yeast phase
- A mixture of yeast cells and pseudo mycelium and true mycelium are seen in Vivo and Nutritionally poor media.

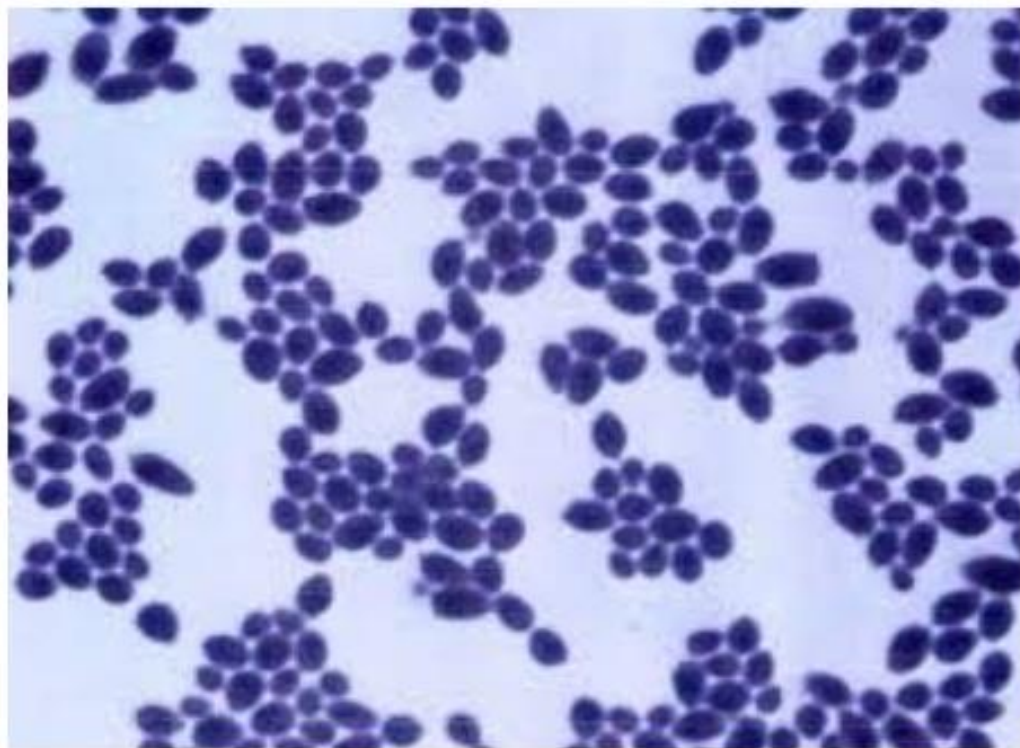
STRUCTURE OF C.ALBICANS



Macroscopic and Microscopic appearance of *Candida* spp



GRAM STAIN; CANDIDA YEAST



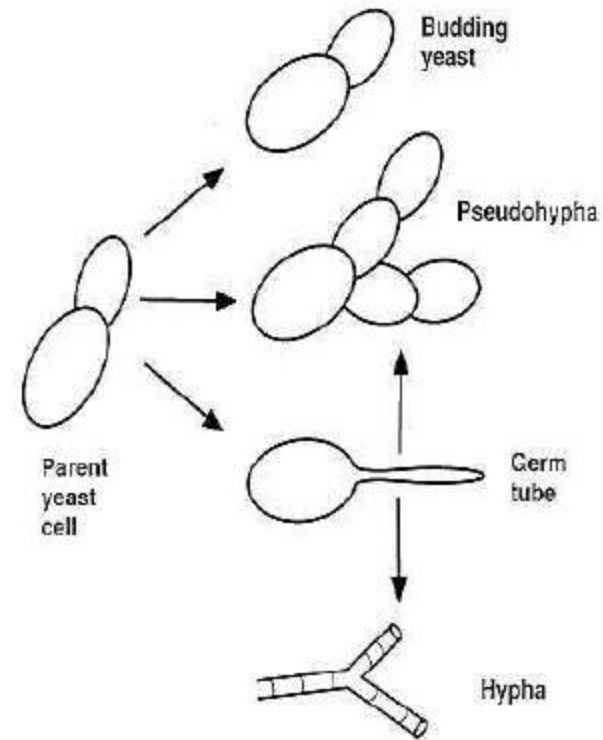
Pseudohyphal structures in Candida

Candida albicans - Corn
meal agar, MMPC-UTMB



Important species of *Candida* in Human infections

- *C.albicans*
- *C.tropicalis*
- *C.glabrata*
- *C.Krusei*



Easier Identification of species as C.albicans

- ***Germ tube test***
identifies C.albicans from other Candida species.
- Majority of Diagnostic laboratories depend on this test.



Procedure of Germ Tube Test

Put 0.5 ml of sheep or human serum into a small tube.

Note: Fetal bovine serum can also be used instead of human serum.

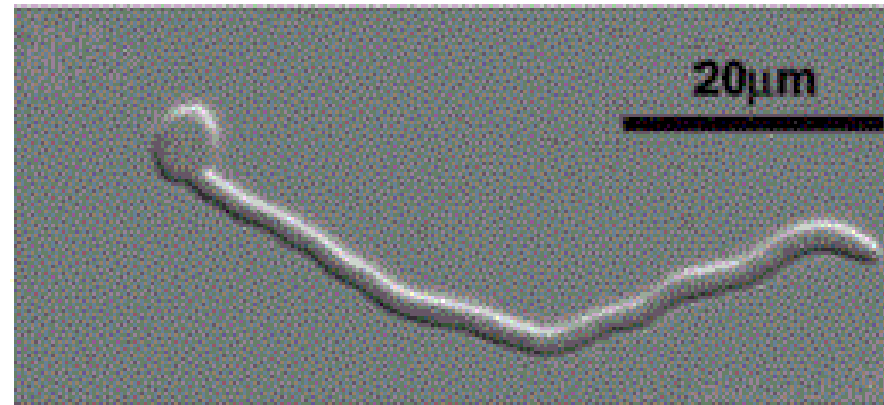
Using a Pasteur pipette, touch a colony of yeast and gently emulsify it in the serum.

Note: Too large of an inoculum will inhibit germ tube formation.

Incubated the tube at 37°C for 2 to 4 hours.

Transfer a drop of the serum to a slide for examination.

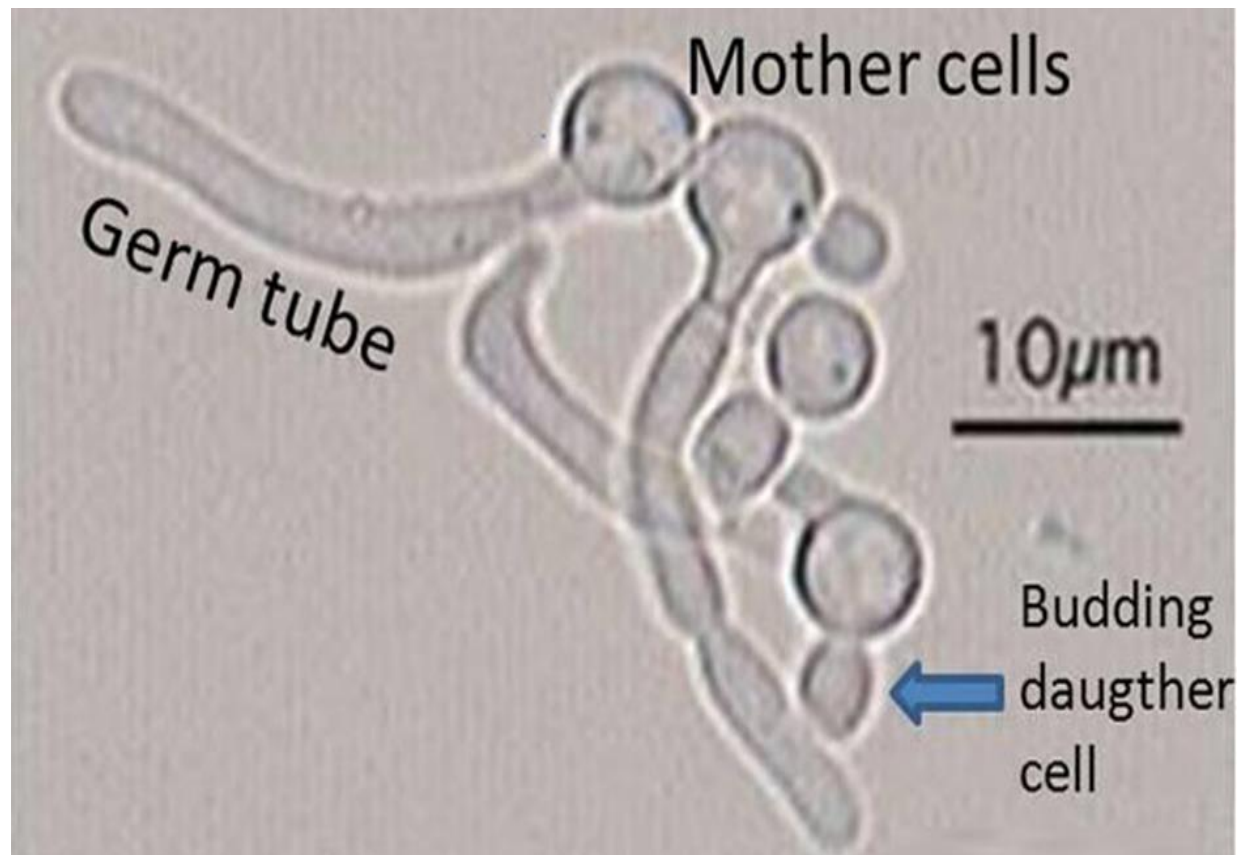
Coverslip and examine microscopically under low and high power objectives.



Germ Tube (no constriction)



Pseudohyphae (constriction)



- Candidiasis is a fungal infection that can affect areas such as the:
- Skin
- Genitals
- Throat
- Mouth
- Blood



EPIDEMIOLOGY

- Over 75% of women suffer from a *C. albicans* infection, usually vulvovaginal candidiasis, in their lifetimes, and 40-50% of them will have additional occurrences(s).
- Interestingly, *C. albicans* are the 4th leading cause for nosocomial infections in patients' bloodstreams.
- This could result in an extremely life-threatening, systemic infection in hospital patients with a mortality rate of 30%

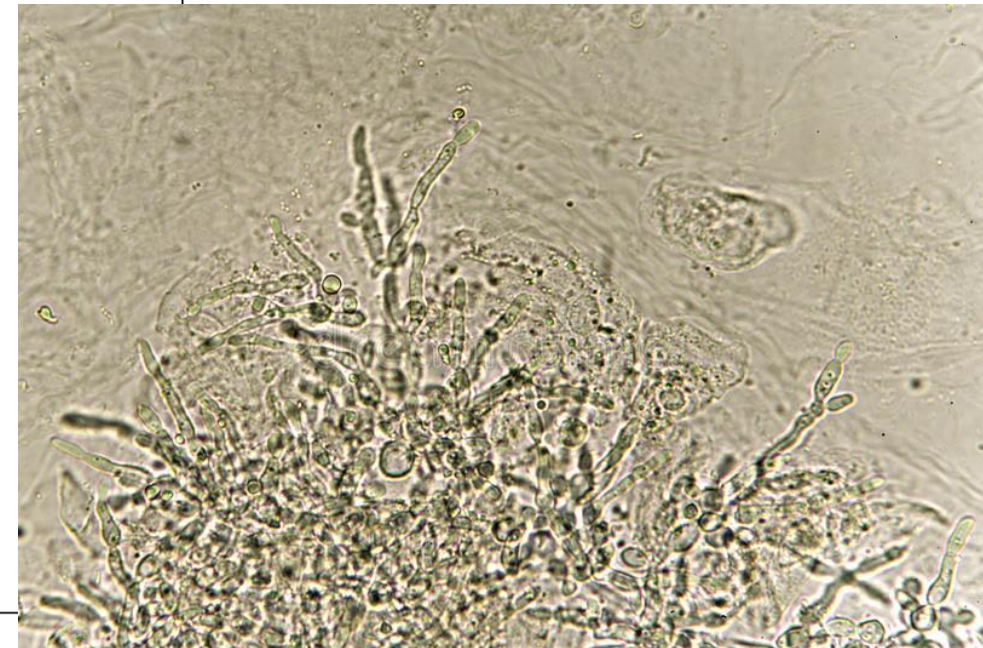
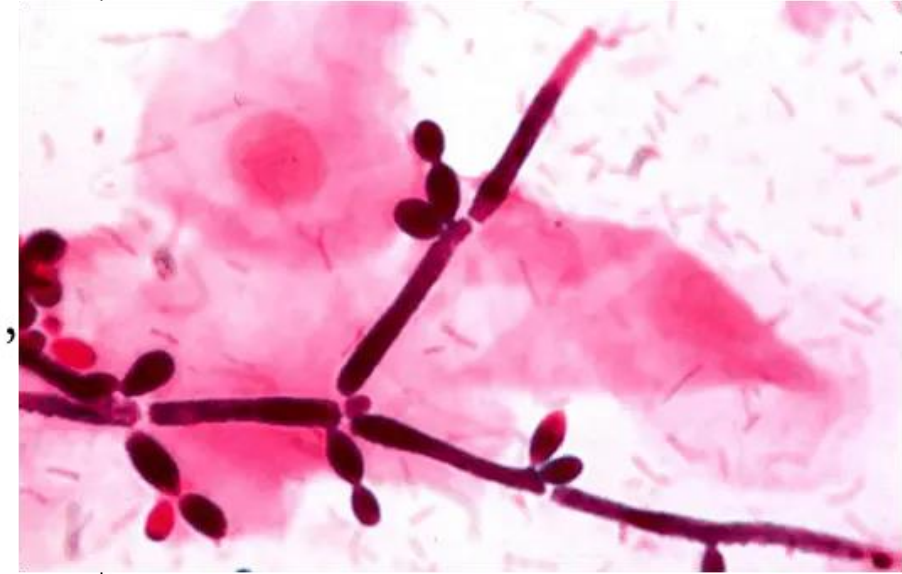
Laboratory diagnosis of *Candida albicans*

Specimen: It is according to the site of the lesion **i.e.** exudates, vaginal, oral swab, tissue, nail scrap, blood.

1. Microscopic Examination

1- Stained smears: With Gram stain, yeast cells are seen as Gram positive, oval budding yeast cells attached to pseudohyphae.

2- 40% KOH: budding yeast, pseudohyphae.



Laboratory diagnosis of *Candida*

Culture

Isolation of *Candida*

- Difficult to isolate if the infection is localized in internal organ
- Sample: Blood, CSF, tissue biopsies
- Media: SDA with antibiotics

Identification

- Similar as with mucocutaneous candidiasis



Laboratory diagnosis of *Candida*

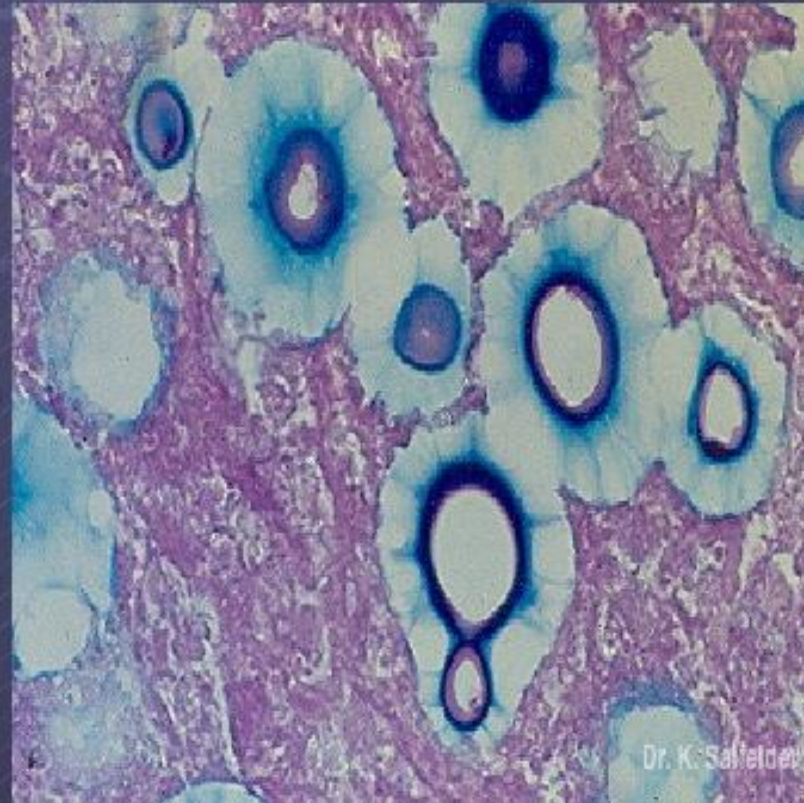
Immunodiagnosis

Antigen detection

- Important diagnostic tool, particularly in immunocompromised patients
- Techniques: LA and ELISA
- Detection in serum, urine and other body fluids
- Antigens: mannan, an undefined glycoprotein, a 47 kDa protein, enolase

Cryptococcus neoformans

- A Capsulated yeast – A true yeast..
- A sporadic disease in the past.
- Most common infection in AIDS patients.



Dr. K. S. S. S.

Culturing

- CSF -Culturing on Sabouraud's agar, and incubated at 37° c for upto to 3 weeks
- Cultures appear as Creamy, white, yellow Brown colored
Simple urease test helps in confirming the isolate.

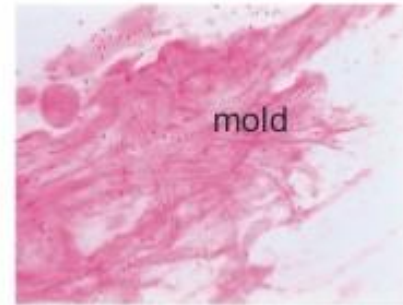
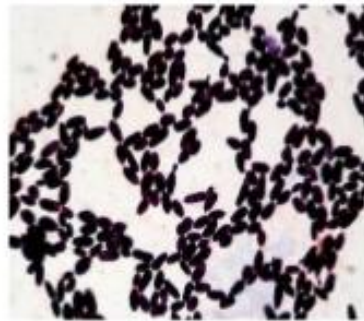


Direct Exams used to identify fungi directly from patient specimens

- **Gram stain** – all specimen types can be Gram stained. Can only reliably detect yeast by Gram stain.
- **KOH preparation** – Skin, Hair or Nails examined for both yeast and/or hyphae
- **Calcofluor white stain** – all specimen types can be stained and examined for yeast and/or hyphae
- **India ink** – Primarily used for CSF for the detection of *Cryptococcus neoformans* and *C. gattii*

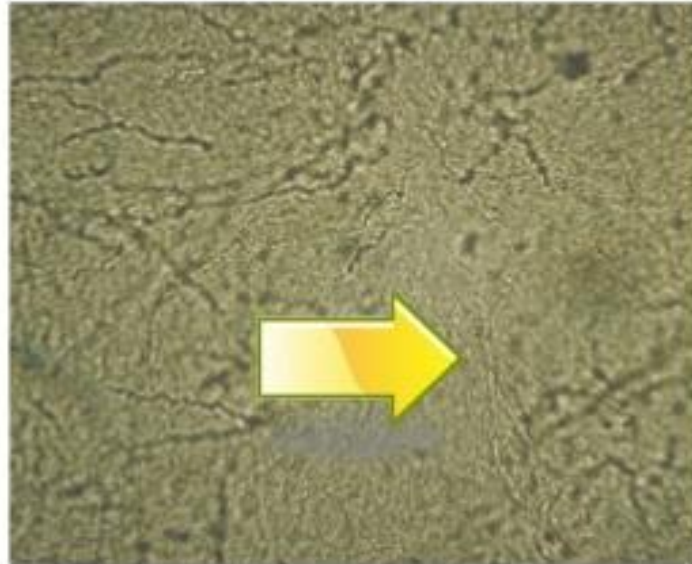
Gram Stain

Yeast cells stain blue [Gram positive]. Examine for budding cells to confirm that it is a yeast cell and not an artifact. Examination on oil immersion lens. You can also detect pseudohyphae on Gram stain. Mold can be difficult to identify on a Gram stain.

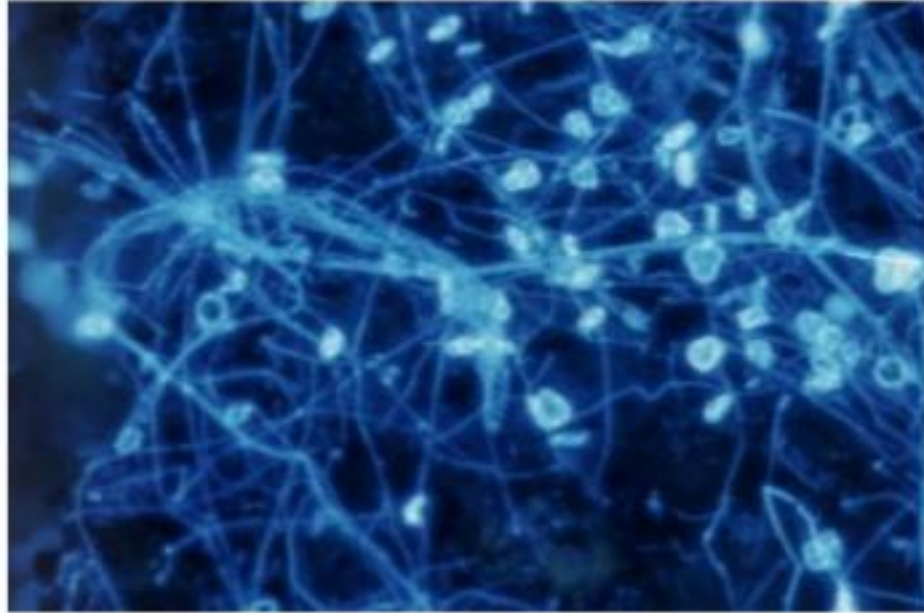


KOH – potassium hydroxide prep

Used to detect yeast and/or hyphae in skin, hair and nail specimens using 40X light microscope.
KOH dissolves keratin found in cell material
and frees hyphae from the cell
KOH exams can be difficult to interpret!



Calcofluor white stain



Yeast, pseudohyphae, and mycelial fungi bind with the Calcofluor white stain.

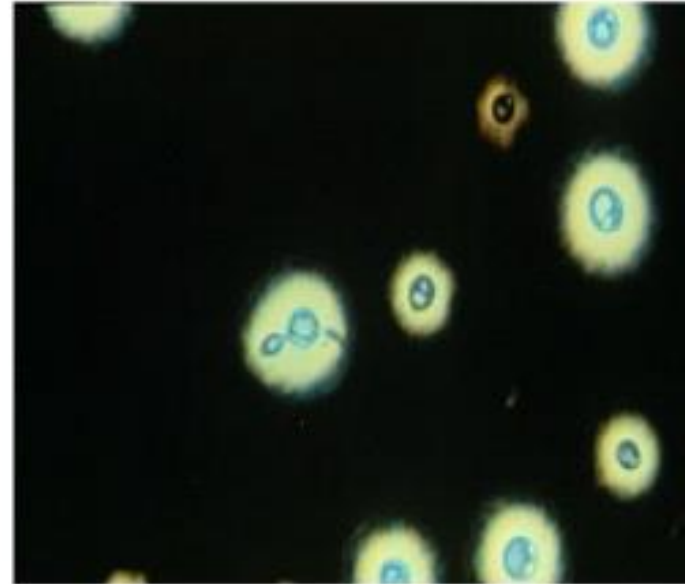
Prep is interpreted using a fluorescence microscope. Sensitivity and specificity is improved over the KOH preparation.

India Ink

One drop of black ink is placed into one drop of CSF and examined using a 40X lens on light microscope

It is a “negative” stain, staining the background not the yeast cell

The clearing is the polysaccharide capsule of *Cryptococcus neoformans* or *C. gattii*. Specificity is improved if you look for budding yeast cells.



Membrane function

Amphotericin B

Cell Wall Synthesis

Caspofungin

**Nucleic acid
synthesis**

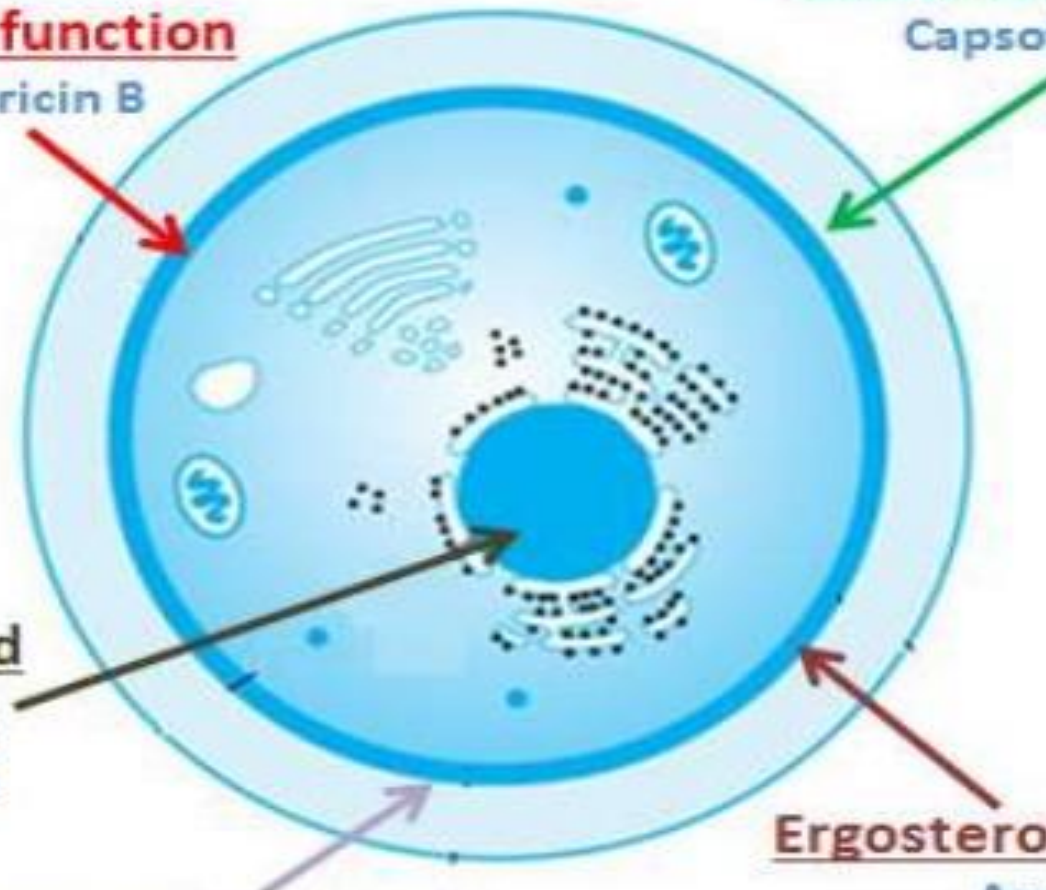
Flucytosine

Ergosterol synthesis

Azoles

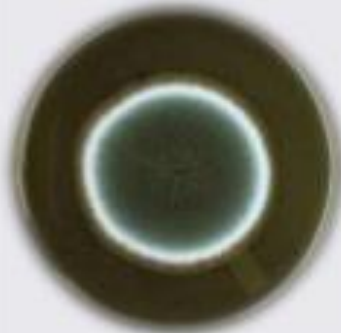



Lanosterol synthesis

Terbinafine





ASPERGILLOSIS

	<i>A. fumigatus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. terreus</i>
Macroscopic morphology	<ul style="list-style-type: none"> • Velvety or powdery • At first white than turning dark greenish to gray with a narrow white border. • Reverse white to tan 	<ul style="list-style-type: none"> • Wooly • At first white to yellow than turning black • Reverse white to yellow 	<ul style="list-style-type: none"> • Velvety • Yellow to green or brown • Reverse goldish to red brown 	<ul style="list-style-type: none"> • Usually velvety • Cinnamon brown • Reverse white to brown
Picture				

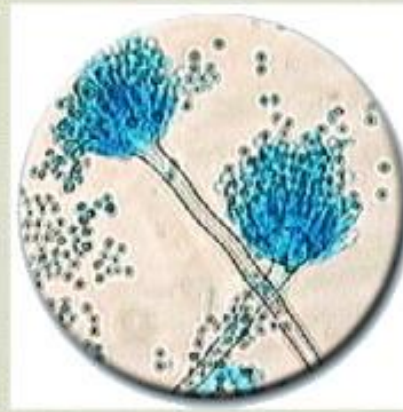
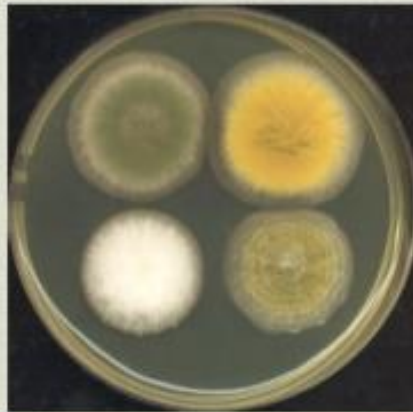
ASPERGILLOSIS

- ◆ One of the largest of the fungal genera
- ◆ Hundred of species have been recorded
- ◆ The most important species:

- ◆ *A. fumigatus*

- ◆ *A. flavus*

- ◆ *A. niger*



CLASSIFICATION OF ASPERGILLOSIS

Airways/nasal
exposure to
airborne
Aspergillus

Persistence
without disease
- colonisation of
the airways or
nose/sinuses

Invasive aspergillosis

- Acute (<1 month course)
- Subacute/chronic necrotising (1-3 months)

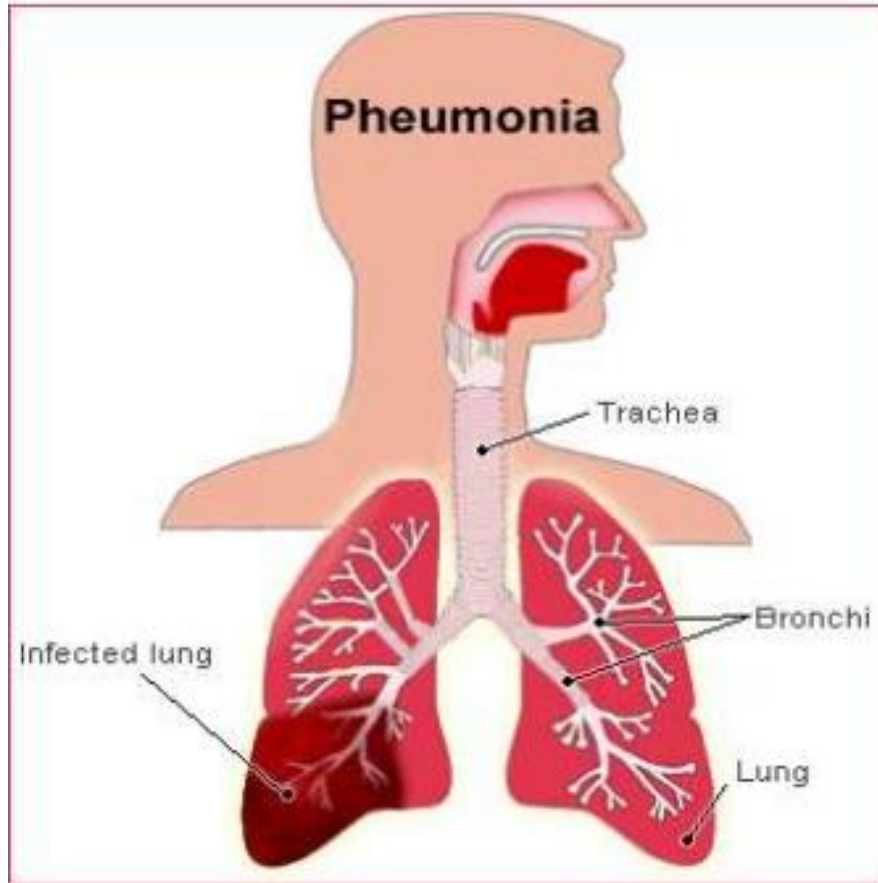
Chronic aspergillosis (>3 months)

- Chronic cavitary pulmonary
- Aspergilloma of lung
- Chronic fibrosing pulmonary
- Chronic invasive sinusitis
- Maxillary (sinus) aspergilloma

Allergic

- Allergic bronchopulmonary (ABPA)
- Extrinsic allergic (broncho)alveolitis (EAA)
- Asthma with fungal sensitisation
- Allergic Aspergillus sinusitis (eosinophilic fungal rhinosinusitis)

Aspergilloma



- Fungus colonize preexisting (Tuberculosis) cavities in the lung and form compact ball of mycelium which is later surrounded by dense fibrous wall presents with cough, sputum production
- Haemoptysis occurs due to invasion of blood vessels
- Cases of aspergilloma rarely become invasive

Laboratory diagnosis

Specimens

Sputum, other respiratory specimens, lung biopsy specimens

Microscopic examination

KOH mount

The fungus appears as non-pigmented septate mycelium, 3-5 μm in diameter with characteristic dichotomous branching and an irregular outline

Rarely the characteristic sporing heads of *Aspergillus* species are present

Culture

- *Aspergillus* species grow readily on SDA without cycloheximide at 25-37°C
- Colonies appear after 1-2 days of incubation
- Species are identified according to the morphology of their conidial structures

Skin tests

- Skin tests with *Aspergillus* species antigen are useful for the diagnosis of allergic broncho pulmonary aspergillosis

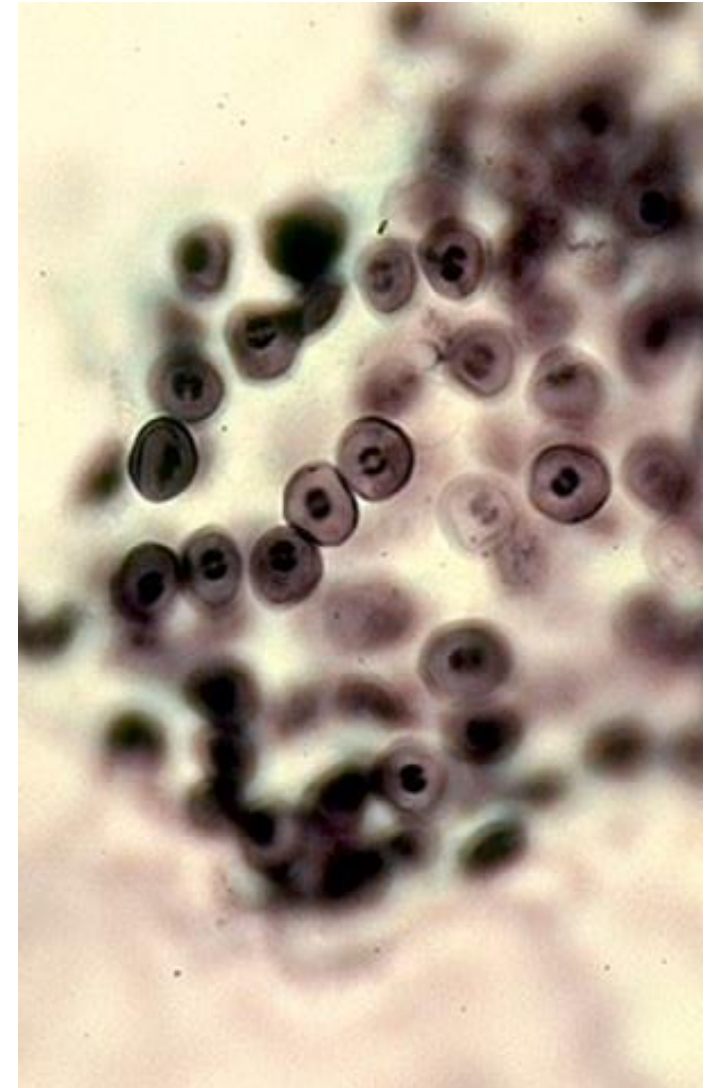
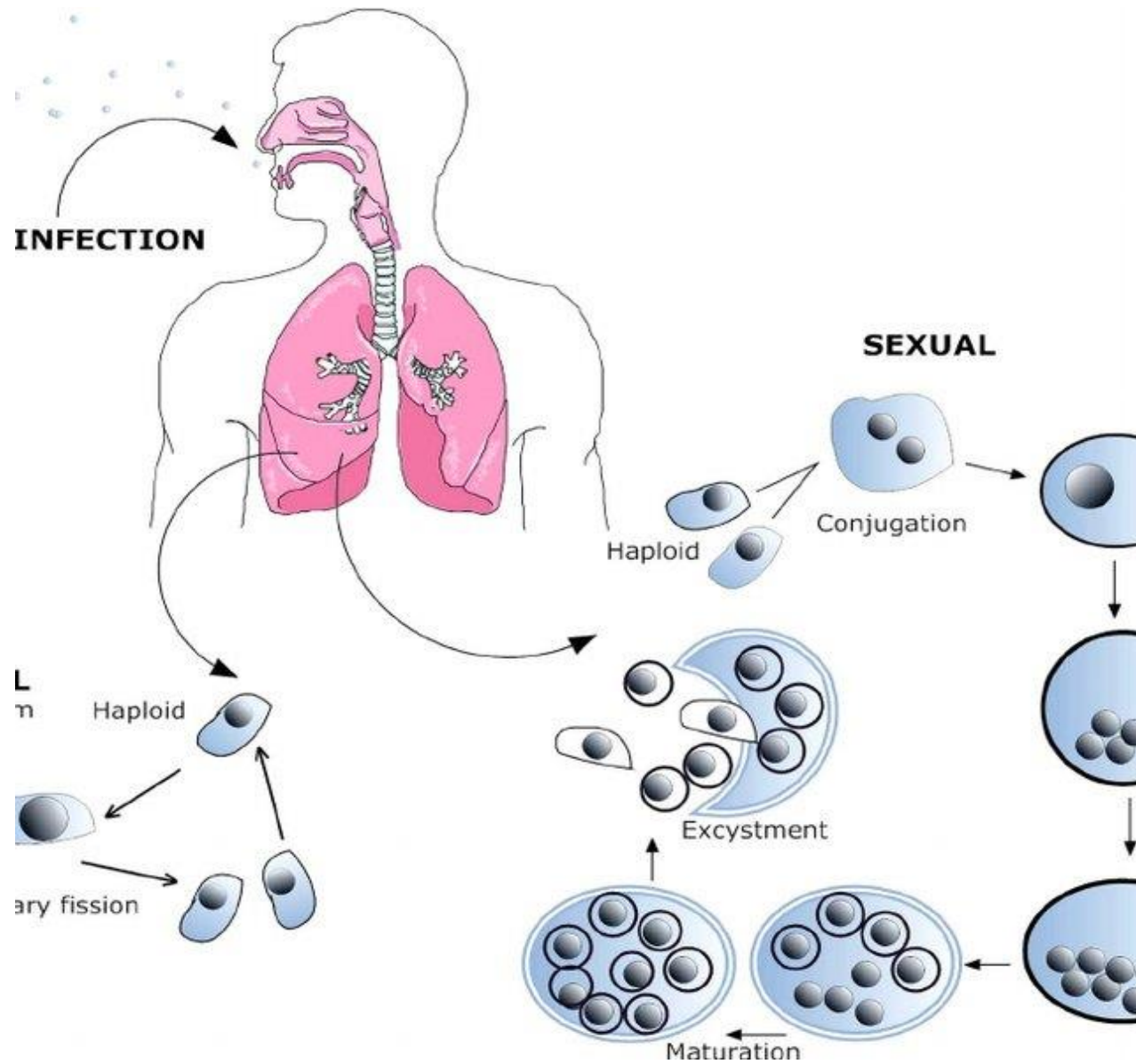
Aspergillosis Treatment Options

- ❖ Amphotericin B
- ❖ Amphotericin B lipid formulations
- ❖ Itraconazole
- ❖ Voriconazole

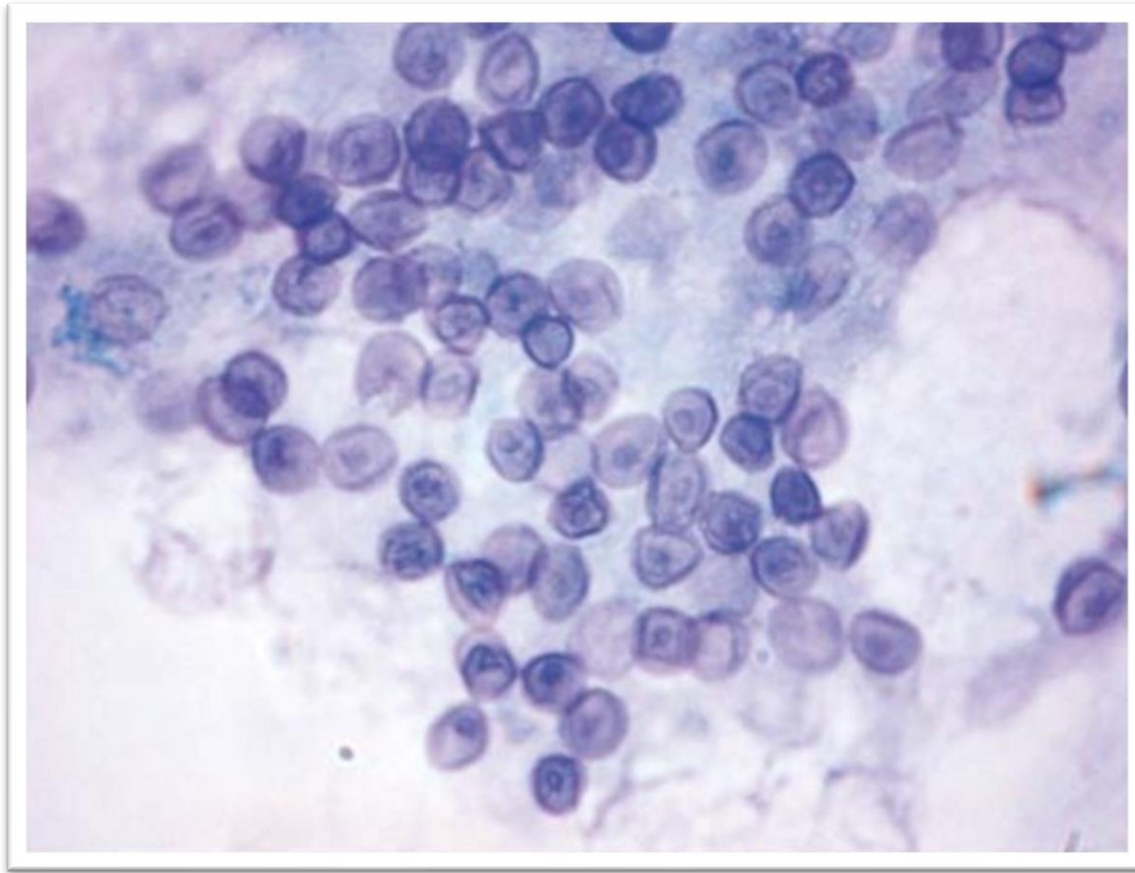
Pneumocystis jiroveci

- *Pneumocystis jiroveci* causes pneumonia in immunocompromised individuals.
- Microorganisms of the genus *Pneumocystis* were previously considered protozoa. However, molecular-biological and genetic studies have shown that it is an ascomycete-like fungus.
- However, they are similar to protozoa in morphology and other properties, sensitivity to antimicrobial drugs, and it is possible that these are the transitional microorganisms between protozoa and fungi.

Pneumocystis jiroveci



Pneumocystis jiroveci



Pathogenetic features of pneumocystosis pneumonia

- In cases of immunodeficiency other than AIDS, interstitial pneumonitis with plasmatic cells develops as a result of infiltration of the intercellular areas of the alveoli with plasmatic cells.
- In pneumocystosis pneumonia observed during AIDS, there are no plasmatic cells, the blockade of gas exchange in alveoli causes respiratory failure.
- P.jiroveci is an extracellular parasite. In the lung tissue, the extracellular parasite aggregates tightly to the alveolar epithelium, and their growth is usually limited by the surfactant layer on the surface of the alveolar epithelium.
- P.jiroveci does not cause disease in people without immunodeficiency

Microbiological diagnostics

- It is possible to detect trophozoites and cyst forms in smears prepared from bronchial lavage and sputum, stained with Giemsa, silvering, as well as with toluidine.
- In smears stained by the Giemsa method, the cytoplasm of the fungus is blue, and the nucleus is red-purple.
- It is also possible to detect the originators in patches by means of IFR.
- *P.jirovecii* is a non-cultivable microorganism, so the cultural method is not used.
- *P.jirovecii* is part of the obligate microflora of the human body, so the detection of antibodies in the blood serum is not of diagnostic importance.
- Treatment is carried out with trimethoprim sulfometaxazol (biseptol) and pentamidine.

The causative agents of mycotoxicoses

- Many mold fungi distributed in soil and plants synthesize toxic substances - mycotoxins. Mycotoxins are secondary metabolites of these fungi and are thermostable substances. Their accumulation in food products contaminated with mold fungi causes food poisoning in humans and animals - *mycotoxicoses*. The producers of mycotoxins are mainly grains, rice, corn, etc. Although it contaminates cereals, it can also be found in other food products. Mycotoxins accumulate during harvesting, storage and processing of agricultural plants and food products under unfavorable conditions.

Forms of mycotoxicoses:

- ***Aflatoxicosis*** is a mycotoxicosis caused by aflatoxins mainly synthesized by *Aspergillus flavus*. Aflatoxins are widely distributed in plant foods. Aflatoxins do not decompose during thermal processing and have high toxicity. Acute poisoning caused by aflatoxins in animals is characterized by slowness of movement, convulsions, paresis, hemorrhages, edema, and liver damage, and has high lethality. Necrosis, cirrhosis, and primary cancer develop in the liver.

Forms of mycotoxicoses:

- ***Fusariotoxicoes*** are mycotoxicoses caused by mycotoxins of *Fusarium* fungi. The development of the *Fusarium sporotrichiella* fungus on cereals causes the accumulation of mycotoxins in them. the use of such cereals in food causes mycotoxicosis - alimentary-toxic alekia. As a result, the amount of granulocytes in the blood decreases sharply, and then acute damage to myeloid and lymphoid tissues, bone marrow necrosis develops, which leads to a violation of hematopoiesis.
- The mycotoxin of the *Fusarium graminearum* fungus has a neurotropic effect and causes mycotoxicosis - **"drunk bread"** syndrome with symptoms such as weakness, impaired gait, acute headaches, dizziness, vomiting, diarrhea, abdominal pain.

Forms of mycotoxicoses:

- *Ergotism* occurs when cereal crops, mainly rye, are damaged by the fungi *Claviceps purpurea* and *Claviceps paspalum*. Mycotoxins of ryegrass consist of alkaloids of lysergic acid and clavine alkaloids, which have neurotoxic effects.
- The acute form of ergotism is accompanied by gastroenteritis and neurological symptoms - paresthesias and convulsions, often resulting in death. The chronic form is accompanied by symptoms of polyneuritis, vomiting, gastrointestinal disturbances.