

A stack of books is shown from a low angle, with the spines of several books visible. The books have various colored covers, including yellow, red, and white. A semi-transparent gradient overlay, transitioning from orange to red, is positioned over the right side of the books. The title 'OPPORTUNISTIC MYCOSES' is written in white, bold, uppercase letters on this overlay.

# OPPORTUNISTIC MYCOSES

Dr. Tanmay Mehta



## Opportunistic mycoses

- **Caused by**
  - Fungi normally a part of human anatomical flora (e.g. *Candida*) or
  - Found in nature and frequently isolated as laboratory contaminants (e.g. *Aspergillus*, *Rhizopus* and *Penicillium*)
- **Causing infection in presence of opportunities such as low immunity**



## CANDIDIASIS

- Yeast like fungus that produces pseudohyphae
- **Species of *Candida*:**
  - *Candida albicans*: most common and most pathogenic
  - Other *Candida* species which can occasionally cause infection such as—*C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. dubliniensis*, *C. kefyr*, *C. guilliermondii* and *C. viswanathii*



## Pathogenesis

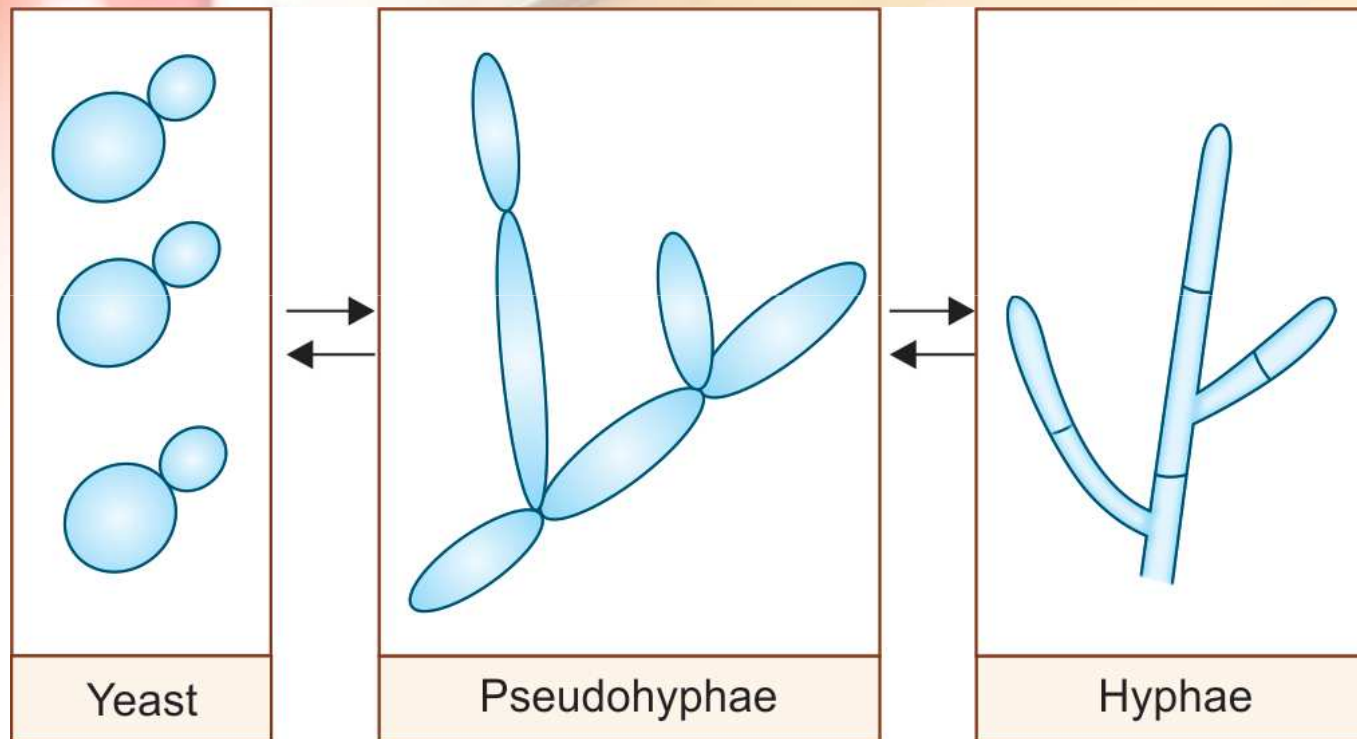
- Worldwide in distribution. Most common fungal infection
- **Predisposing Factors**
  - **Physiological state:** Extremes of age (infancy, old age), pregnancy
  - **Low immunity:** steroid or immunosuppressive drugs, post-transplantation, malignancy, HIV-infected people
  - **Patients on broad spectrum antibiotics**
  - **Others:** Diabetes mellitus, febrile neutropenia and zinc or iron deficiency



## Virulence Factors

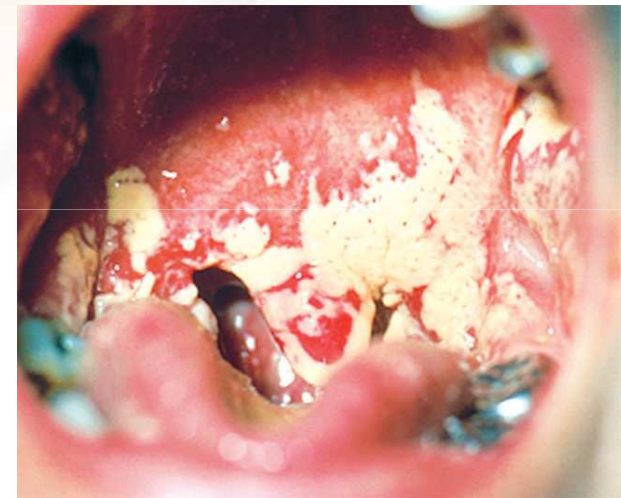
- **Adhesins**
- **Enzymes** - aspartyl proteinases and serine proteinases - tissue invasion
- **Toxins:** Glycoprotein extracts of Candida cell wall are pyrogenic
- **Pseudohyphae:** Presence of pseudohyphae indicates active infection
- **phenotypic switching** - Ability to transform between three phenotypic forms in the tissue - yeast (blastospores), pseudohyphae, and true hyphae

# Phenotypic switching of *Candida*



## Clinical Manifestations

- **Mucosal candidiasis:**
  - Oropharyngeal candidiasis : white, adherent, painless patch
  - Vulvovaginitis: pruritus, pain, and vaginal discharge (whitish curd like in severe cases)
  - Balanitis and balanoposthitis
  - Esophageal candidiasis





## Clinical Manifestations

- **Chronic mucocutaneous candidiasis**
  - Infants and children with deficient CMI
  - Lesions on hair, nail, skin, and mucous membrane
  - Usually resistant to treatment
- **Cutaneous candidiasis:**
  - Intertrigo: erythema and pustules in the skin folds; associated with tight fitting undergarments and sweating



## Clinical Manifestations

- Paronychia and onychomycosis
- Diaper candidiasis
- Perianal candidiasis
- Erosio interdigitalis blastomycetica.
- Generalized disseminated cutaneous candidiasis





## Clinical Manifestations

- **Invasive candidiasis:** hematogenous or local spread
  - Urinary tract infection
  - Pulmonary candidiasis, Septicemia
  - Arthritis and osteomyelitis, Meningitis
  - Ocular—keratoconjunctivitis and endophthalmitis
  - Hepatosplenic candidiasis, Disseminated candidiasis
  - Nosocomial candidiasis (mainly by *C. glabrata*).

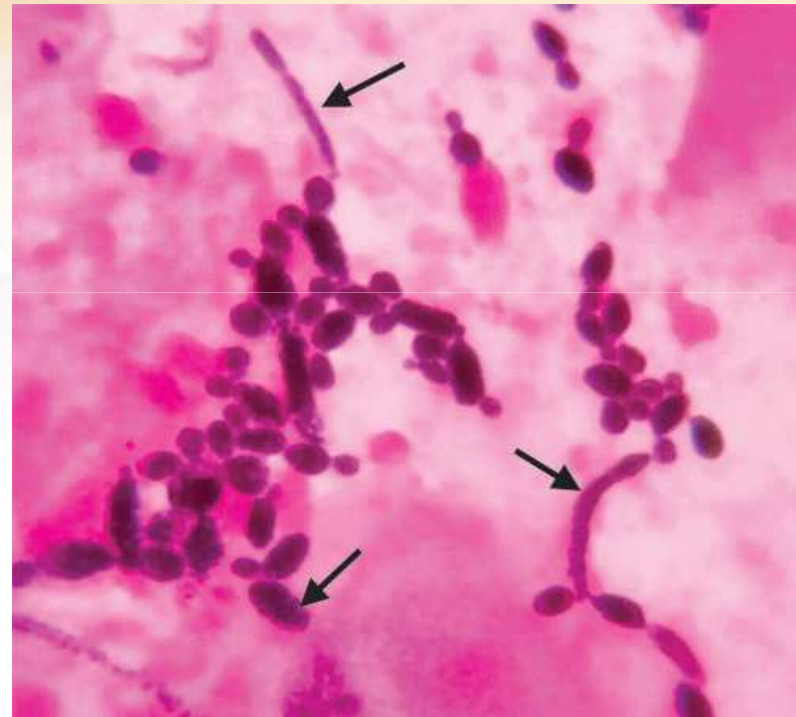


## Clinical Manifestations

- **Allergic candidiasis includes:**
  - **Candidid:** allergic reaction to metabolites of *Candida*
    - vesicular lesions in the web space of hands and other areas
  - Similar dermatophytid reaction
- **Other allergic reactions include:** Gastritis, irritable bowel syndrome and eczema

## Laboratory Diagnosis

- **Specimen Collection**
- whitish mucosal patches, skin and nail scrapings, sputum, urine or blood
- **Direct Microscopy**
  - Gram staining - gram-positive oval budding yeast cells with pseudohyphae



## Culture

- SDA with antibiotic supplements at 37°C
- Grow in bacteriological culture media -blood agar
- Blood culture bottles (conventional and automated blood)
- Colonies - creamy white, smooth, and pasty with typical yeasty odor



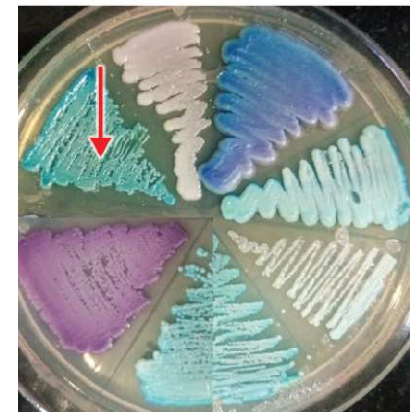
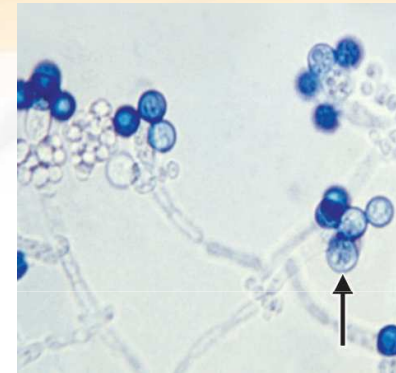
## Tests for Species Identification

- **Germ tube test:** specific test for *C. albicans*
- Also called Reynolds Braude phenomenon
- **Germ tubes** - long tube like projections extending from the yeast cells
- Differentiated from pseudohyphae as there is no constriction at the origin



## Tests for Species Identification

- **Dalmau plate culture:** Culture on cornmeal agar → *C. albicans* produces thick walled chlamydospores
- **CHROMagar:** Different *Candida* species produce different colored colonies





## Tests for Species Identification

- **Growth at 45°C:**
  - It differentiates *C. albicans* (grows) from *C. ubliniensis* (does not grow at 45°C)
- **Carbohydrate fermentation test**
- **Carbohydrate assimilation**
- **Molecular methods**





## Immunodiagnosis

- **Antibody detection:** ELISA, latex agglutination tests - antibodies against cell wall mannan antigen
- **Antigen detection:** cell wall mannan and cytoplasmic antigens - ELISA
- **Enzyme detection:** *enolase*, *aspartate* proteinase
- **Test for metabolites:** mannitol, arabinitol
- G test is done for detection of  $\beta$ -1-3 -D-glucan



## Treatment

- Cutaneous candidiasis or oral thrush: topical azole
- Esophageal and vulvovaginal candidiasis: oral fluconazole or caspofungin
- Disseminated candidiasis: liposomal amphotericin B or caspofungin
- *C. glabrata* and *C. krusei* exhibit intrinsic resistance to azoles



## CRYPTOCOCCOSIS

- *Cryptococcus neoformans*
- **Species and Serotypes**
- Two species: *C. neoformans* and *C. gattii* and four serotypes A, B, C and D.
- Two varieties—*C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans*



## Pathogenesis

- Infection is acquired by inhalation of aerosolized forms of *Cryptococcus*
- Immunocompetent individuals - defense mechanisms limit the infection
- Low immunity - pulmonary infection → dissemination through blood
- **CNS spread:** cross blood-brain barrier - migrate directly across the endothelium or carried inside the macrophages as “Trojan horse”



## Virulence factors

- Polysaccharide capsule
  - Antiphagocytic and also inhibits the host's local immune responses
- Ability to make melanin by enzyme phenyl oxidase
- Other enzymes – phospholipase and urease



## Risk factors

- Patients with advanced HIV infection with CD4 T cell counts less than  $200/\mu\text{L}$
- Patients with hematologic malignancies
- Transplant recipients
- Patients on immunosuppressive or steroid therapy



## Clinical Manifestations

- **Pulmonary cryptococcosis:** first and the most common presentation
- **Cryptococcal meningitis:** chronic meningitis, with headache, fever, sensory and memory loss, cranial nerve paresis and loss of vision (due to optic nerve involvement)
- **Skin lesions**
- **Osteolytic bone lesions**



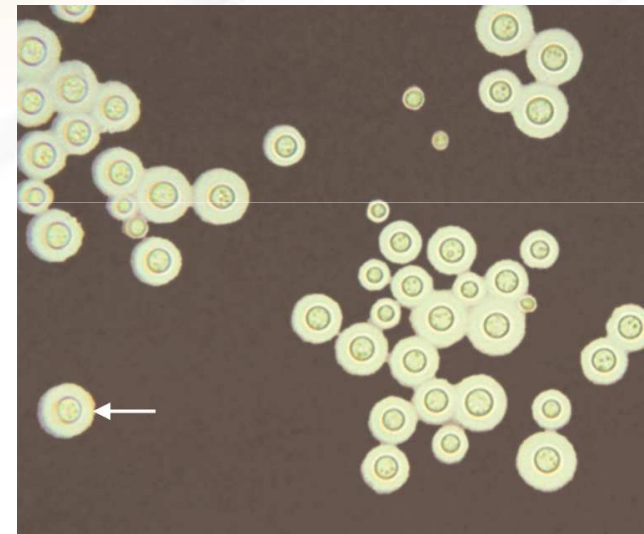
## Epidemiology

- **Geographical distribution:** *C. neoformans var. grubii* (serotype A) strains are found worldwide
- *C. neoformans var. neoformans* (serotype D) strains are restricted to Europe
- *C. gattii* is confined to tropics
- **Habitat:** *C. neoformans* – soils contaminated with avian excreta and pigeon droppings.



## Laboratory Diagnosis

- **Specimens** - CSF, blood or skin scrapings
- **Direct Detection Methods**
- **Negative staining:** Modified India ink stain and nigrosin stain - demonstrate the capsule
- **Gram staining** - gram-positive round budding yeast cells





## Cryptococcus

- **Other stains:**
  - Mucicarmine stain: It stains the carminophilic cell wall of *C. neoformans*
  - Masson-Fontana stain: It demonstrates the production of melanin
  - Alcian blue stain to demonstrate the capsule.
- **Capsular Antigen detection:** from CSF or serum by latex agglutination test

## Culture

- SDA without antibiotics, blood agar or chocolate agar and incubated at 37°C
- Blood inoculated in biphasic blood culture bottles
- Colonies - mucoid creamy white and yeast like
- **Confirmation of *Cryptococcus species* :**
  - Niger seed agar and bird seed agar
  - Growth at 37°C
  - Urease test is positive
  - Assimilation of inositol and nitrate
  - Mouse pathogenicity test



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## Treatment

- Without CNS involvement: Fluconazole
- HIV-infected patients with CNS involvement: induction phase for two weeks (amphotericin B — flucytosine) → oral fluconazole therapy till CD4 T cell count raises  $>200 /\mu\text{L}$  for 6 months



# ZYGOMYCOSIS

- Life-threatening infections caused by aseptate fungi belonging to the phylum Zygomycota
  - 1. Order mucorales (causes mucormycosis)**
    - *Rhizopus* (*R. arrhizus* and *R. microsporus*)
    - *Mucor racemosus*, *Rhizomucor pusillus*
    - *Lichtheimia corymbifera*
    - *Apophysomyces elegans*.
  - 2. Order entomophthorales (causes entomophthoromycosis)**
    - *Basidiobolus ranarum*
    - *Conidiobolus coronatus*.



# Mucormycosis

- **Pathogenesis**
- Spores found ubiquitously in the environment
- Transmission - inhalation, inoculation or rarely ingestion of spores
- Spores → mycelial form which are angioinvasive
- **Predisposing factors:**
  - Conditions with increased iron load
  - Diabetic ketoacidosis
  - End stage renal disease
  - Iron therapy or deferoxamine
  - Defects in phagocytic functions

# Clinical Manifestations

## 1. Rhinocerebral mucormycosis:

- Most common form, Orbital cellulitis, proptosis and vision loss

## 2. Pulmonary mucormycosis - in patients with leukemia

## 3. Cutaneous mucormycosis

## 4. Gastrointestinal mucormycosis – necrotizing enterocolitis

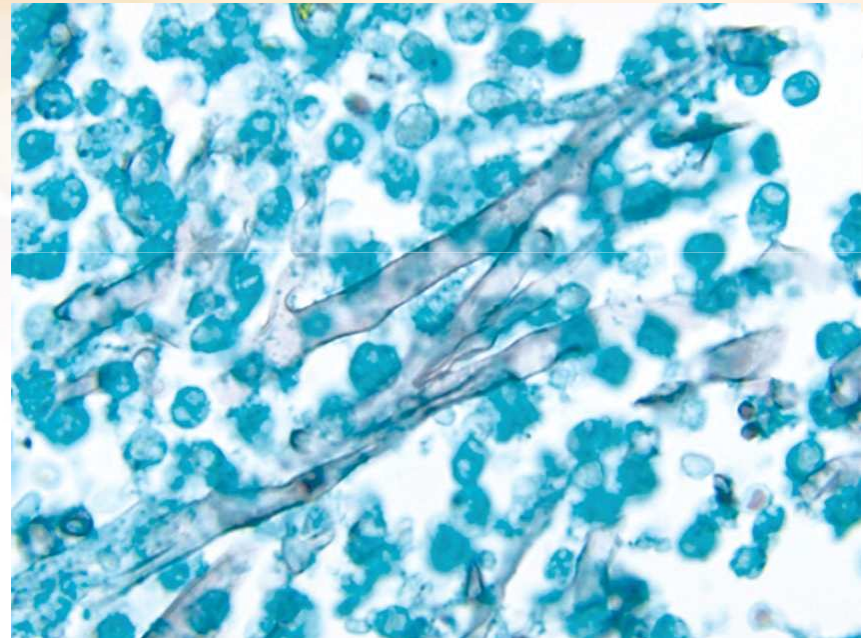
## 5. Disseminated mucormycosis: Brain

## 6. Miscellaneous forms



## Laboratory Diagnosis

- **Histopathological staining or methenamine silver stain of tissue biopsies shows broad aseptate hyaline hyphae with wide angle branching**





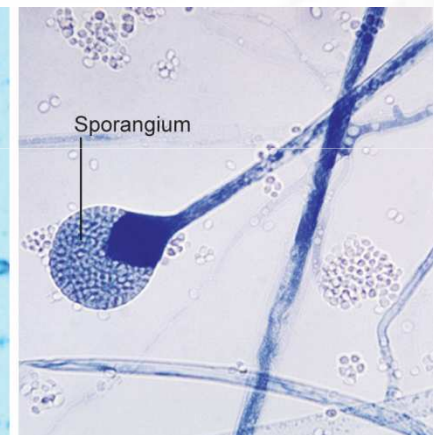
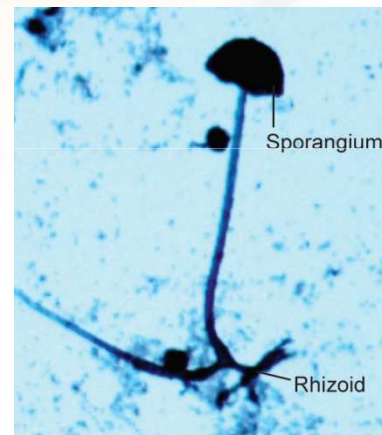
## Laboratory Diagnosis

- **Culture on SDA at 25°C:** white cottony woolly colonies with tube filling growth (**lid lifters**)
- **In some species (e.g. *Rhizopus*) - salt and pepper appearance**



## Laboratory Diagnosis

- **LPCB mount colonies** - broad aseptate hyaline hyphae → sporangiophore → sporangium containing numerous sporangiospores
- **Rhizoids** - root like growth arising from hyphae



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## Treatment

- Amphotericin B deoxycholate - drug of choice for all forms of mucormycosis
- Alternatives - Posaconazole or isavuconazole



## ASPERGILLOSIS

- Aspergillosis refers to the invasive and allergic diseases caused by a hyaline mold named *Aspergillus*. There are nearly 35 pathogenic and allergenic species of *Aspergillus*, important ones being—*A. fumigatus*, *A. flavus* and *A. niger*



## Pathogenesis

- Widely distributed in nature - decaying plants
- Transmission – inhalation
- **Risk factors for invasive aspergillosis are:**
  - Glucocorticoid use (the most important risk factor)
  - Profound neutropenia
  - Neutrophil dysfunction
  - Underlying pneumonia, chronic obstructive pulmonary disease, tuberculosis or sarcoidosis
  - Anti-tumor necrosis factor therapy.



## Clinical Manifestations

- **Pulmonary aspergillosis:** most common form
  - Allergic bronchopulmonary aspergillosis (ABPA)
  - Severe bronchial asthma
  - Extrinsic allergic alveolitis
  - Aspergilloma (fungal ball)
  - Acute angioinvasive pulmonary aspergillosis
  - Chronic cavitary pulmonary aspergillosis



## Other forms of aspergillosis

- Invasive sinusitis
  - Chronic granulomatous sinusitis
  - Maxillary fungal ball
  - Allergic fungal sinusitis
- Cardiac aspergillosis: Endocarditis (native or prosthetic) and pericarditis
- Cerebral aspergillosis: Brain abscess, hemorrhagic infarction, and meningitis



## Other forms of aspergillosis

- Ocular aspergillosis: Keratitis and endophthalmitis
- Ear infection: Otitis externa
- Cutaneous aspergillosis
- Nail bed infection: Onychomycosis
- Mycotoxicosis

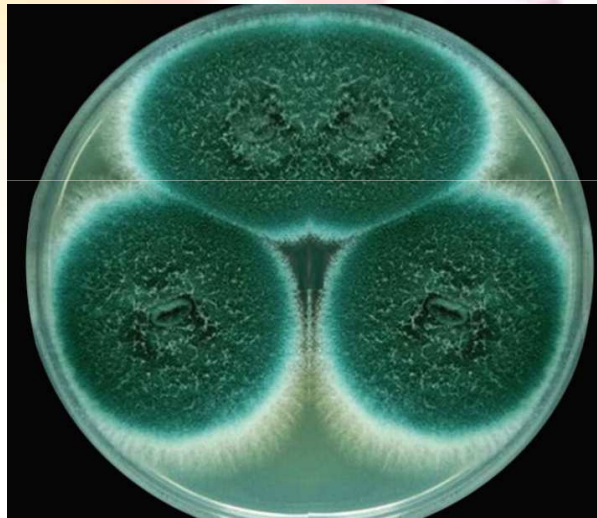




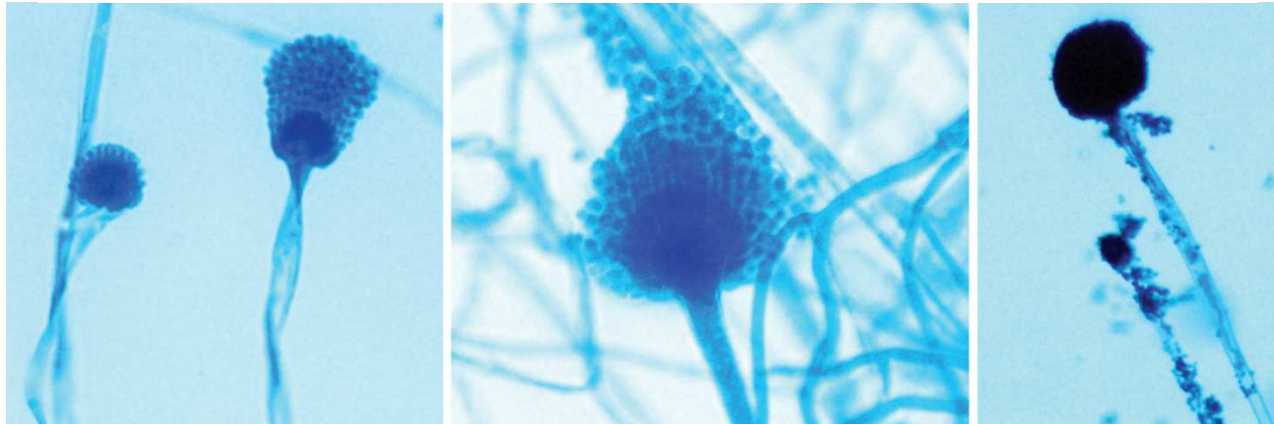
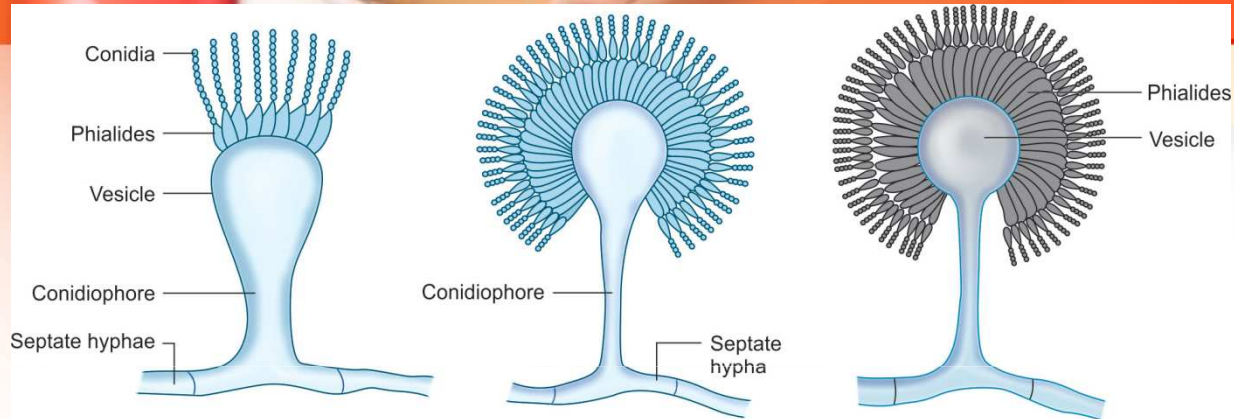
## Laboratory Diagnosis

- Specimens - sputum and tissue biopsies
- **Direct Examination**
  - KOH (10%) mount or histopathological staining of specimens → narrow septate hyaline hyphae with acute angle branching
- **Culture:** SDA and incubated at 25°C
  - Species identification is done based on macroscopic and microscopic (LPCB mount) appearance of the colonies

# Colony morphology of common Aspergilli



# Microscopy of common Aspergilli



## Identification features of *Aspergillus species*

Aspergillus	Macroscopic appearance of colony	Microscopic appearance of colony (LPCB mount)
<i>A.fumigatus</i>	<b>Colonies-</b> Smoky green, velvety to powdery, reverse is white	Vesicle is conical-shaped. Phialides are arranged in single row Conidia arise from upper third of vesicle Conidia are hyaline
<i>A.flavus</i>	<b>Colonies-</b> Yellow green, velvety, reverse is white	Vesicle is globular shaped Phialides in one or two rows Conidia arise from entire vesicle Conidia are hyaline
<i>A.niger</i>	<b>Colonies-</b> Black, cottony type, reverse is white	Vesicle is globular shaped Phialides in two rows Conidia arise from entire vesicle Conidia are black



## Other Tests

- **Antigen Detection** - ELISA - *Aspergillus* specific galactomannan
- **Antibody Detection**
  - Useful for chronic invasive aspergillosis and aspergilloma, where the culture is usually negative
  - In allergic syndromes such as ABPA and severe asthma, specific serum IgE levels are elevated.
- **Detection of Metabolites**
  - b-1-3-D-glucan (by G test) or mannitol (by gas liquid chromatography)



## Treatment Aspergillosis

- Invasive aspergillosis—voriconazole
- ABPA—itraconazole
- Single aspergilloma—surgery
- Chronic pulmonary aspergillosis—itraconazole or voriconazole
- For prophylaxis - posaconazole



## PENICILLIOSIS

- **Clinical Significance**
- *Penicillium* has more than 250 species, most are found as saprophytes in the environment
- *Penicillium marneffe*
- Mycotoxicoses - toxins released by certain species of *Penicillium* such as *P. cyclopium*, *P. verrucosum* and *P. puberulum*



## PENICILLIOSIS

- Invasive penicilliosis: endophthalmitis and endocarditis
- Superficial disease: otomycosis, keratitis and Onychomycosis
- Allergic disease: asthma and allergic pneumonitis



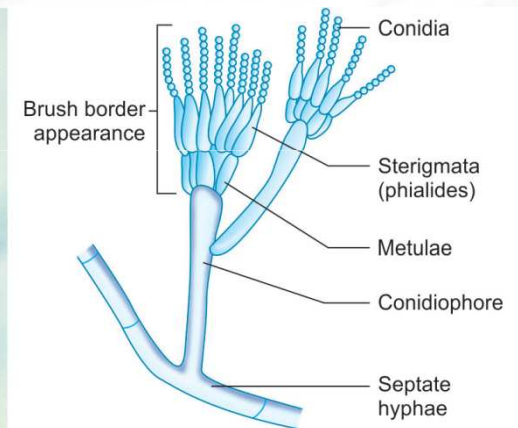
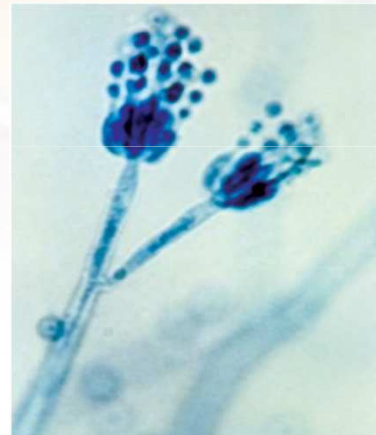
## Laboratory Diagnosis

- *P. marneffei* - dimorphic fungus
- Other *Penicillium* - only as molds, grow easily on SDA at 25°C
- **Colonies** - rapid growing, flat with velvety to powdery texture and greenish in color



## Microscopic appearance

- **LPCB mount of the colonies**
- Hyaline thin septate → conidiophore and its branches → elongated metulae → flask-shaped phialides originate → chain of conidia
- **brush border appearance**



A laboratory setting with a petri dish and pipette. The background is a blurred image of a laboratory bench with a petri dish and a pipette. The title 'Penicillium marneffeii' is overlaid on a red banner at the top right.

## Penicillium marneffeii

- *Penicillium marneffeii* - thermally dimorphic fungus
- *Renamed as -Talaromyces marneffeii*
- causes opportunistic infection in HIV-infected patients. It
- **Epidemiology**
  - Endemic in South East Asian countries including Thailand, Vietnam and India (Manipur)



## Pathogenesis

- Rural areas where the bamboo rats (reservoirs) are prevalent,
- No direct rat to man transmission
- **Risk factors:** Immunocompromised hosts
- **Transmission** - inhalation of conidia
- Mold to yeast conversion occurs in the lungs and then the yeast form spreads via blood to reticuloendothelial system

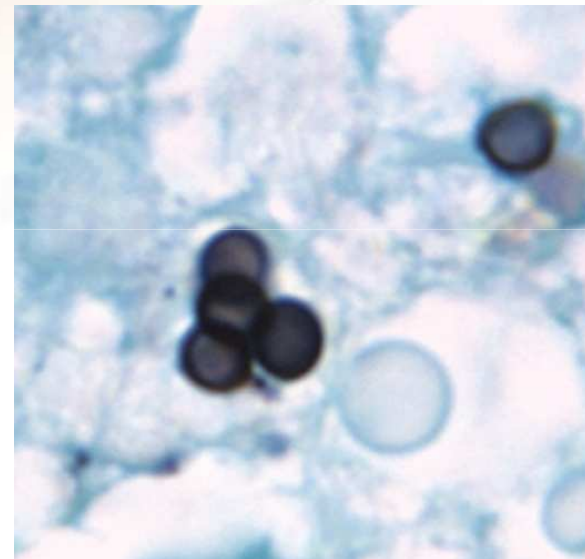


## Clinical Manifestations

- **Systemic infection:** fever, weight loss, dyspnea, lymphadenopathy and hepatosplenomegaly
- **Skin lesions:** Warty lesions mimicking that of molluscum contagiosum

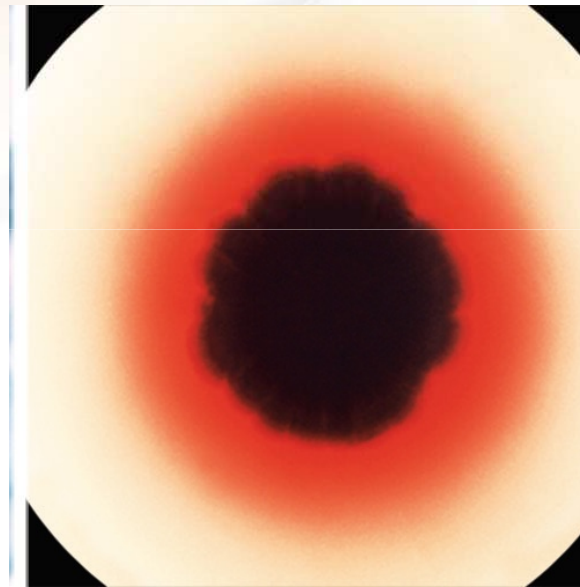
## Laboratory Diagnosis

- **Histopathological staining** of tissue sections, skin scrapings or blood smear
- **Oval or elliptical yeast cells with central septation,**



## Laboratory Diagnosis

- **Culture:** *P. marneffei* being dimorphic
- Yeast like colonies at 37°C and mold form at 25°C
- Mold form - **brick red pigment**





## Treatment

- AIDS patients with severe penicilliosis - amphotericin B → maintenance therapy with itraconazole for 12 weeks
- Mild penicilliosis: Itraconazole is recommended for 12 weeks



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# Pneumocystis Pneumonia

- **Taxonomy**
- Once thought to be a protozoan, now it is classified under fungus based on nucleic acid sequence studies
- Renamed from *P.carinii* to *Pneumocystis jirovecii*

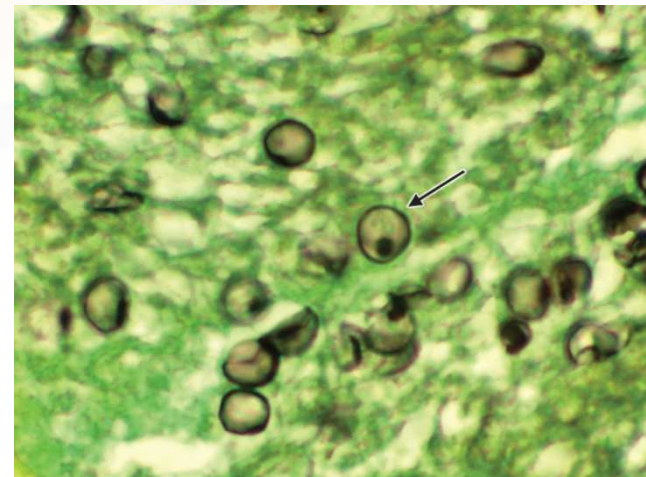


## Pathogenesis

- Exists in cyst and trophozoite forms
- In human tissues both cysts and trophozoites are found.
- Cysts inhaled → carried to the lungs → transform into the trophozoite stage → induce an inflammatory response → recruitment of plasma cells → **frothy exudate** filling the alveoli → **plasma cell pneumonia**

## Laboratory Diagnosis

- **Specimens:** lung tissue or fluids obtained by bronchoscopy, bronchial lavage, or open lung biopsy
- **Histopathological examination**
- Gomori's methenamine silver staining - method of choice





## Laboratory Diagnosis

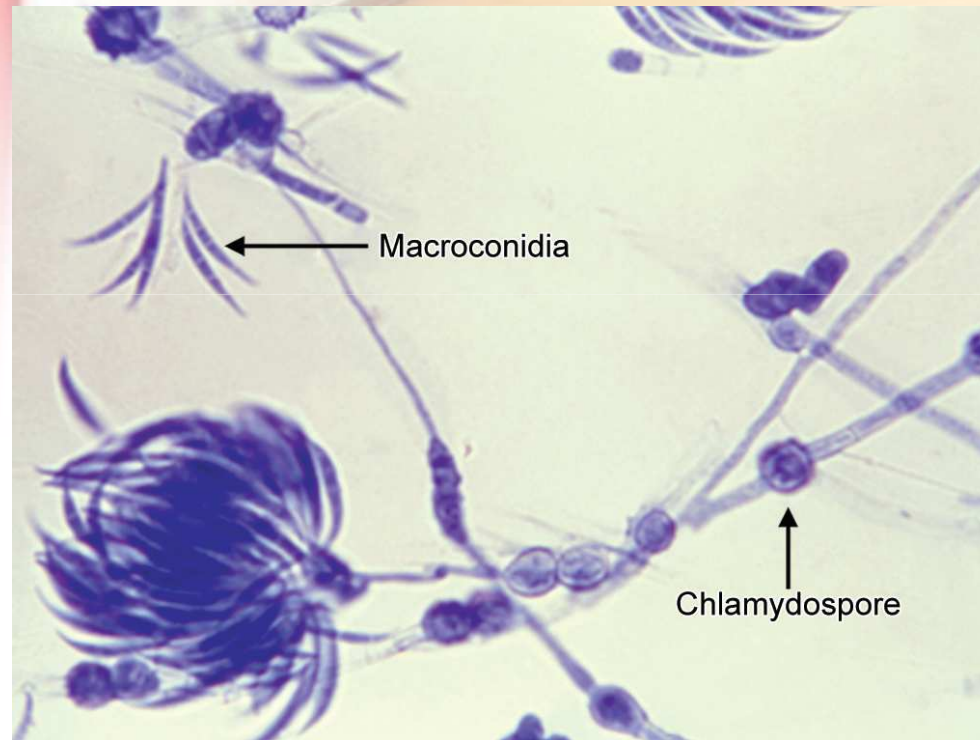
- Cysts resemble black colored **crushed ping-pong balls**, **against the green background**
- PCR assay
- Detection of 1, 3  $\beta$ -D-glucan in serum
- **Treatment:**
  - Cotrimoxazole - 14 days in non-HIV patients and 21 days in patients with HIV
  - Recommended drug for primary and secondary prophylaxis in patients with HIV



## Fusariosis

- Soil and plant saprophytes found worldwide rarely cause human infections
- **In immunocompetent individuals:**
  - Keratitis in contact lens wearers
  - Onychomycosis.
- **In immunocompromised patients** - angioinvasive and cause pulmonary and sinus infection
- Neutropenia and hematologic malignancies - disseminated fusariosis

## *Fusarium species (LPCB mount)*





## Laboratory Diagnosis

- **Humans pathogens:** *F. solani* (most common), followed by *F. oxysporum* and *F. verticillioides*
- **Colony:** Rapid growing Cottony, flat, spreading white to pink colonies
- **LPCB mount** - hyaline septate hyphae bearing round microconidia, sickle-shaped large macroconidia and chlamydospores
- **Treatment**
- Liposomal amphotericin B, voriconazole or posaconazole
- Resistant to many antifungal agents