# FUNGI

Fungal Classification, Structure, and Replication

# Fungal Taxonomy, Structure, and Replication

- Kingdom Fungi.
- eukaryotic
- rigid cell wall
- chitin and glucan
- cell membrane:

- **ergosterol** is substituted for cholesterol (major sterol component)

# Fungal Taxonomy, Structure, and Replication

- unicellular or multicellular
- **yeasts** (**pseudohyphae )** or **Molds – hyphae (filamentous, hairy,** or **woolly)**
- Yeast (C. albicans) - one cell, asexual reproduction by budding (blastoconidia) or by division. They can produce filamentous structueres resembling molds – pseudohyphae, elongated cells resembling sausages

- Molds(hyphae) – elongation at both ends, can be multinucleated – coenocitic or septated

- **Hyphae - apical extension,**
  - **coenocytic** (hollow and multinucleate)
  - **septate** (divided by partitions or cross-walls)

  **Mycelium - vegetative hyphae**
  - **aerial hyphae - conidia**

**DIMORPHIC FUNGI**

# Fungal Morphology

**Hypae** (threads)
making up a **mycelium**

**Yeasts**

Many pathogenic fungi are **dimorphic**, forming hyphae at ambient temperatures but yeasts at body temperature.

# Fungal Taxonomy, Structure, and Replication

- Asexual spores : **sporangiospores** and **conidia**
- Sporangiospores - **sporangia**
- Conidia - borne naked

# Mycoses

- **Superficial mycoses** - limited to the keratinized outermost layers of the skin and hair shafts
  (asymptomatic, cosmetic significance - pigments produced by the fungi-black, brown, green,
  DO NOT elicit an immune response because the fungi colonize tissues that are nonliving).
- **Cutaneous mycoses**-restricted to the keratinized layers of the skin and its appendages (hair and nails), **Dermatophytes**
  (various immune responses may be evoked)
- **Subcutaneous mycoses** - dermis, subcutaneous tissues, muscle, fascia, bones(rare)
  saprophytic fungi - they live in soil and on vegetation

# Superficial Mycoses

| Disease | Causative organisms |
| --- | --- |
| Pityriasis versicolor<br>Seborrhoeic dermatitis including Dandruff and Follicular pityriasis | *Malassezia* spp.<br>(a lipophilic yeast) |
| Tinea nigra | *Hortaea werneckii* |
| White piedra | *Trichosporon* spp. |
| Black piedra | *Piedraia hortae* |

# Cutaneous Mycoses

- **Dermatophytoses - are caused** by the agents of the genera:
  - *Epidermophyton,*
  - *Microsporum,* and
  - *Trichophyton*
- The dermatophytoses are characterized by an anatomic site-specificity according to genera:

**- *Epidermophyton floccosum*** - infects only skin and nails, but does not infect hair shafts and follicles.

**- *Microsporum* spp**. - infect hair and skin, but do not involve nails.

**- *Trichophyton* spp**. - may infect hair, skin, and nails.

- **Dermatomycoses** - are cutaneous infections due to other fungi, the most common of which are ***Candida* spp.**

**LAB.DG.** Specimens are mounted in a **drop of 10% to 20% KOH** on a glass slide and examined microscopically.

**Tinea capitis** - after cleaning the selected area with spirit hair are plucked by sterile forceps(Wood's lamp)
- hair brush technique - suspected scalp infections

**Onychomycosis** – nails, decontaminated with 70% alcohol, from the deeper part of the discolored or dystrophic parts of the nails.

three or more specimens collected at different times from the same patient

**Culture** - dermatophyte test medium, mycobiotic agar, Sabouraud's glucose agar

**TREATMENT -** Topical agents include azoles (miconazole, clotrimazole, econazole, tioconazole, and itraconazole), terbinafine, and haloprogin

# Subcutaneous Mycoses

**-Lymphocutaneous sporotrichosis** - *Sporothrix schenckii* –

**-Chromoblastomycosis** - *Phialophora verrucosa, Cladosporium carrionii*

- **Subcutaneous zygomycosis** - *Basidiobolus haptosporus* –

- Lab. Dg.- Skin scrapings from cutaneous lesions; sputum and needle biopsies from pulmonary lesions; nasal discharges, scrapings and aspirates from sinuses in patients with rhinocerebral lesions; and biopsy tissue from patients with gastrointestinal and/or disseminated disease

Th.: potassium iodide, ketoconazole, itraconazole

# Subcutaneous Mycoses

- **Subcutaneous phaeohyphomycosis** – *Exophiala, Phialophora, Cladophialophora , Phaeoannellomyces*

**-Eumycotic mycetoma** - *Acremonium sp., Aspergillus nidulans, Madurella grisea, Madurella mycetomatis, Scedosporium apiospermum*

**!Three Bacterial genera !**

*Actinomyces species*

*Nocardia species*

*Streptomyces species*

- fungi infections i.e Mycetoma

**Lab. Dg.: Direct Microscopy:** Serosanguinous fluid containing the granules should be examined using 10% KOH

# Systemic fungal infections

Natural immunity is high; physiologic barriers include:

1. Skin and mucus membranes
2. Tissue temperature-fungi grow better at less than 37°C
3. Redox potential- *in vivo* conditions too reducing for most fungi

## Infection requires a large inoculum and a susceptible host

1. infection often occurs in *endemic areas*
2. most infections are *asymptomatic* or self-limiting
3. in immune-compromised hosts, infections are more often fatal (AIDS)

## Systemic fungal disease is most often associated with four organisms

1. *Coccidioides immitis*
2. *Histoplasma capsulatum*
3. *Blastomyces dermatitidis*
4. *Paracoccidioides brasiliensis (S. America)*

# *Coccidioidomycosis*

*Coccidiodes immitis* is considered to be the *most virulent* of fungal pathogens.

Grows in the soil, but inhalation of a single spore can initiate infection.

**Encounter:** Mycelium found in dry, dusty soil. Contact by **inhalation** of arthroconidia

**Spread:** Most commonly an asymptomatic self limited pulmonary disease, but may spread via the blood to  skin, soft tissues, bones, joints and meninges.

***Symptoms***

1. *Fever*
2. *Arthralgia*
3. *Erythema nodosum*

Exam: Suppurative or granulomatous inflammation

2. Histopathology: spherules or endospores seen in sputum, exudates or tissue
3. Culture: —danger, highly infectious!
4. Serology: Complement fixation assay (in cerebrospinal fluid

**Treatment**

1. Often none.
2. Amphotericin B, azoles

# PARACOCCIDIOIDOMYCOSIS

*Paracoccidioides brasiliensis*

chronic granulomatous disease that characteristically produces a primary pulmonary infection, often inapparent, and then disseminates to form ulcerative granulomata of the buccal, nasal and occasionally the gastrointestinal mucosa.

Lab.Dg.: Skin scrapings, sputum and bronchial washings, cerebrospinal fluid, pleural fluid and blood, bone marrow, and tissue biopsies from various visceral organs.

# *Histoplasmosis*

(also called *cave disease*)

Histoplasmosis is characterized by:
intracellular growth of the pathogen in macrophages,
granulomatous reaction in tissue.

These granulomatous foci may reactivate and cause dissemination of fungi to other tissues.

1.Lung--bronchial obstruction and inflammatory sequelae

2.Disseminated histoplasmosis-fulminant disease that may result in toxic shock

3.CNS-fatal if untreated.

4.Mediastinal fibrosis (rare)

Lab.dg.: Direct histology and culture of blood or bone marrow

Serological testing for antibody and histoplasma antigen in blood and urine.

Urine test: in HIV-infected patients with disseminated histoplasmosis, histo. antigen detection in urine is at least 90% sensitive.

# Blastomycosis

Granulomatous mycotic infection

- predominantly involves lungs and skin;
- can spread to other organs.
- Most prevalent in males 40-60 years of age and children.

Dimorphic organism originates in the soil and infection ensues by inhalation of spores.
Converts to yeast in animal hosts or at 37° *in vitro.*

- **Encounter**: Most cases are in southern, central, and southeastern USA. Infection is by inhalation of spores.
- **Spread:** The pulmonary infection is either self -limited or progressive. Dissemination often occurs to the skin and to the bone - 80% of patients have large skin lesions; a large number also have granulomatous pulmonary lesions.
- **Risk Factors:** Occupational contact with soil; owning a dog. Living in endemic area.
- **Diagnosis:** based on clinical findings and microscopic detection of organisms in tissue specimens

# Opportunistic Mycoses

do not normally cause disease in healthy people,

cause disease in people with weakened immune defenses (immunocompromised people).

Weakened immune function may occur due to
-inherited immunodeficiency diseases,
-drugs that suppress the immune system (cancer chemotherapy, corticosteroids, drugs to prevent organ transplant rejection),
-radiation therapy,
-infections (e.g., HIV),
- cancer,
-diabetes,
-malnutrition.

The most common infections are:
Aspergillosis
Candidiasis
Cryptococcosis
Pneumocystis
Zygomycosis

# *Cryptococcus neoformans*

**-Encounter**: Organism is ubiquitous and infections occur worldwide

*C. neoformans* recovered in large amounts in pigeon poop. Does not Cause disease in birds.

- Primary site of human infection is the lungs

**-Spread**: *Cryptococcal* meningitis is most common disseminated manifestation. Can spread to skin, bone and prostate.

**-Evasion of defenses**: Yeast cells are resistant to phagocytosis because of capsule.

**-Diagnosis**: Lumbar puncture and microscopic examination of cerebrospinal fluid is diagnostic. Cyrptococcal antigens in CSF and serum. Culture of organisms from blood or CSF

**-Treatment**: Amphotericin B & 5FC. Followed by oral fluconazole.

# Aspergillosis

*Aspergillus fumigatus*, *A. flavus* Genus occur worldwide and contains hundreds of species.

Major portal of entry is the respiratory tract.

Dissemination can occur from the lungs and involve other areas

- brain,
- GI tract
- kidney
- CNS
- nasal-orbital cavities

In immunosuppressed hosts: invasive pulmonary infection, usually with fever, cough, and chest pain.

May disseminate to other organs, including brain, skin and bone.

In immunocompetent hosts: localized pulmonary infection in persons with underlying lung disease.

Also causes allergic sinusitis and allergic bronchopulmonary disease.

# Candidiasis

*C. albicans* is a member of the indigenous microbial flora of humans.

1. Found in the gastrointestinal tract, upper respiratory tract, buccal cavity, and vaginal tract.
2. Growth is normally suppressed by other microorganisms found in these areas.
3. Alterations of gastrointestinal flora by broad spectrum antibiotics or mucosal injury can lead to gastrointestinal tract invasion.
4. Skin and mucus membranes are normally an effective barrier but damage by introduction of catheters or intravascular devices can permit *Candida* to enter the bloodstream.

# Chronic mucocutaneous candidiasis

Chronic mucocutaneous candidiasis (CMC)

-clinical pattern of persistent, severe, and diffuse cutaneous candidal infections.

-These infections affect the skin, nails and mucous membranes.

CMC patients often have defects in cell-mediated immunity

CMC can be controlled by oral azole antibiotics.

# Clinical Specimens
# Candida

- Sputum
- Scrapings from lesions
- Blood smears
- Vaginal discharge
- Urine
- Feces
- Nail clippings

# Pneumocystidiomycetes

- **Lab.Dg.:** Microscopic identification of *P. Pneumocystis carinii* - had been considered a protozoan
- *Pneumocystis jirovecii* - human-derived strain

These fungi are found in the lungs of mammals where they reside without causing overt infection until the host's immune system becomes debilitated.

The symptoms of *Pneumocystis* pneumonia (PCP) include

dyspnea, nonproductive cough, fever.

Extrapulmonary lesions occur in a minority (<3%) of patients, involving most frequently the lymph nodes, spleen, liver, and bone marrow.

Typically, in untreated PCP increasing pulmonary involvement leads to death.

*jiroveci* trophozoites and cysts is performed with stains that demonstrate either the nuclei of trophozoites and intracystic stages or the cyst walls In addition, immunofluorescence microscopy using monoclonal antibodies can identify the organisms with higher sensitivity than conventional microscopy.

**Th.:** trimethoprim sulfamethoxazole (TMP-SMX)

# CONVENTIONAL LABORATORY DIAGNOSIS

- **Conventional Microbiologic Methods** Direct microscopy (Gram, Giemsa, and calcofluor white stains)
- Culture
- Identification
- Susceptibility testing
- **Histopathologic Methods** Routine stains (H&E)
- Special stains (GMS, PAS, Mucicarmine)
- Direct immunofluorescence
- In situ hybridization
- **Immunologic Methods**
- Antibody
- Antigen
- **Molecular Methods**
- Direct detection (nucleic acid amplification)
- Identification
- Strain typing
- **Biochemical Methods** Metabolites
- Cell wall components
- Enzymes

# microscopy

- Direct microscopic examination of clinical specimens such as sputum, biopsy, CSF, and/or skin scrapings provides a rapid and accurate diagnosis of some fungal infections.
- Diagnostic features of fungal pathogens, when stained by various dyes (e.g. asexual spores, hyphae etc), helps in the identification.
- Traditionally, the potassium hydroxide preparation has been the recommended method for the direct microscopic examination of specimens *(specimen is treated with 20% KOH to dissolve tissue material, leaving alkali-resistant fungi intact)*.

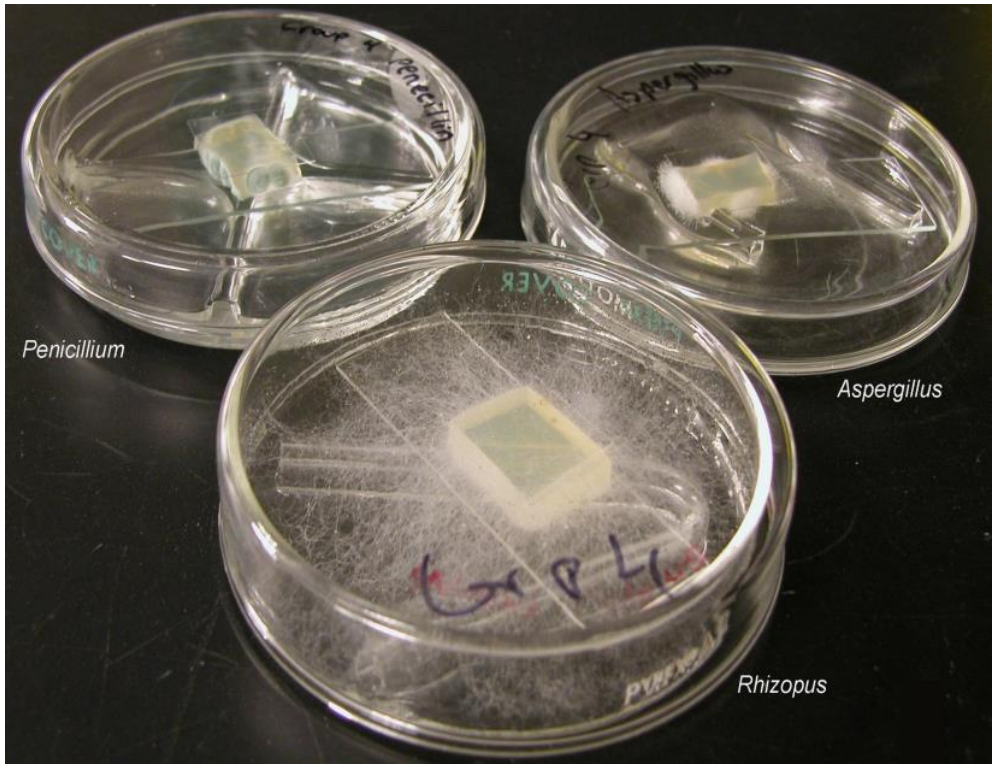
# Culture

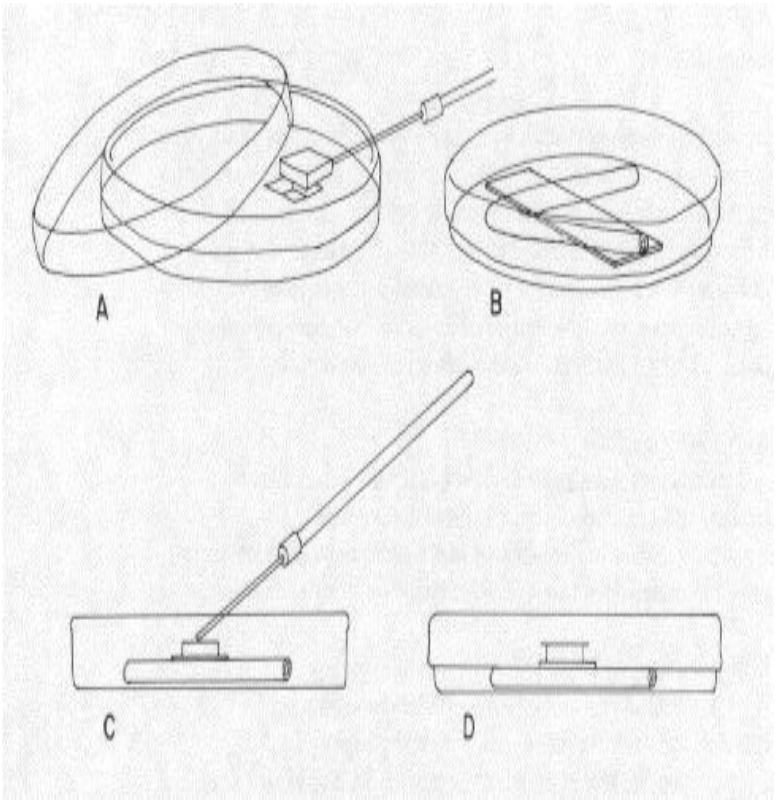
- substrates or chromogens
- direct detection of specific enzymatic activities
- CHROMagar
- selective

# Cultivation media

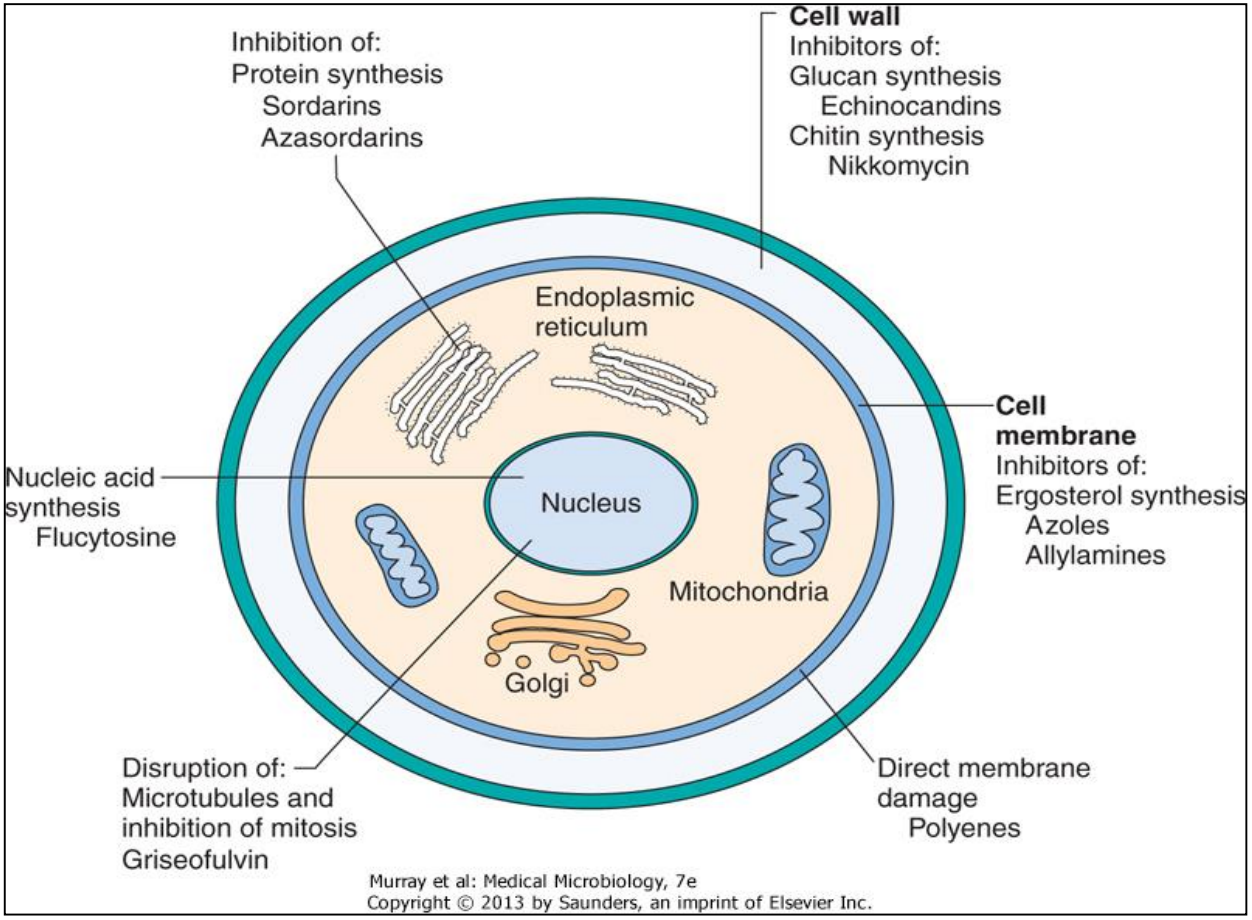
- Selective
- **Sabouraud agar** - dextrose, pepton, less agar, pH 5,5 – acid environment and high concentration of glc
- Saprophytic bacteria and fungi can overgrow pathogenic – addition of chloramphenicol (against bacteria) and cyclohexamid (aganinst saprophytic fungi).
- Recommendation of cultivation on media with and without ATB, always in 25 and 37*C ( some fungi do not grow at 37+ *H. capsulatum)*
- Identification: all are G+, yeat are growing as bacterial colonies, fungi – longer – several days and even weeks, microscopy – rice agar block - morphology

# RICE AGAR



# Antifungal Agents




Target- differences in membrane sterol (ergosterol vs. cholesterol)
- Azoles
- Polyenes
- Allylamines

Target - cell wall biosynthesis (caspofungin)

Target - fungal tubulin (griseofulvin)

Target - fungal nucleoside metabolism. (flucytosine)

Because they are eukaryotic, fungi are biochemically similar to the human host.
Therefore it is difficult to develop chemotherapeutic agents that will destroy the invading fungus without harming the patient.

# Antimycotics

- Polyens - amfotericin B, nystatin
- 
- Azoles – interference with enzymes depending on cytochromes and acting during demetylation of lanosterol to ergosterol - miconazol,ketoconazol, flukonazol,
- 
- Nucleotides – inhibition of DNA a RNA synthesis - 5 fluorocytosin

- Grisanes – inhibition of the function of microtubules
- 
- KJ – activation of lysosomal ensymes

- Longlasting therapy, monitoring of the patient
- Susceptibility testing – not standardised

# ANTIFUNGAL AGENTS

## Topical Antifungal Agents

- Topical treatment is **useful in superficial** fungal infections *confined to the stratum* corneum, squamous mucosa, or cornea, including dermatophytosis (ringworm), candidiasis, tinea versicolor, piedra, tinea nigra, and fungal keratitis.
- **Unsuccessful for mycoses** of the **nails** (onychomycosis) and **hair** (tinea capitis)
- No place in subcutaneous mycoses, such as sporotrichosis and chromoblastomycosis.
- **Efficacy of topical agents** depends not only on the type of lesion and the mechanism of drug action, but also on the viscosity, hydrophobicity, and acidity of the formulation.



## OTHER TOPICAL ANTIFUNGALS

| Drug | Use | Dose |
|---|---|---|
| Tolnaftate | drug for tinea cruris and tinea corporis for 1-3 weeks | 1% lotion/ cream |
| Ciclopirox olamine | newer drug effective in tinea infections, pityriasis versicolor and dermal candidiasis; used for onychomycosis, vaginal candidiasis | 1% cream, 1% topical solution, 1% vaginal cream |
| Undecylenic acid | It is fungistatic. Used for tinea pedis, nappy rash and tinea cruris | |
| Benzoic acid | It has antifungal and antibacterial property. It is fungistatic-weaker than tolnaftate. On hyperkeratotic lesions, it is used in combination with salicylic acid | |
| Butenafine | Efficacy in tinea cruris/ corporis/pedis is similar to that of topical terbinafine | 1% cream; apply locally once/ twice daily. |
| Quiniodochlor | Weak antifungal and antibacterial activity. It has been used for dermatophytosis, mycosis barbae, seborrhoeic dermatitis, infected eczema, furunculosis and pityriasis versicolor | 3% / 4% / 8% cream |
| Sodium thiosulfate | weak fungistatic, active against *Malassezia furfur* | Karpin Lotion 20%. |

**Systemic antifungal therapy should be strongly considered, especially in a patient who is at high risk for disseminated fungal infection, if:**

- Fever persists despite antibacterial agents and negative blood cultures
- High-grade funguria occurs in the absence of a bladder catheter
- Funguria persists after removal of a bladder catheter
- Fungus is cultured from at least two body sites
- Visceral fungal lesions are confirmed

- https://mycology.adelaide.edu.au/mycoses/subcutaneous/
- Murray et all. Medical Microbiology. 7th edition