Mucormycosis and Entomophthoramycosis (Zygomycosis)

By

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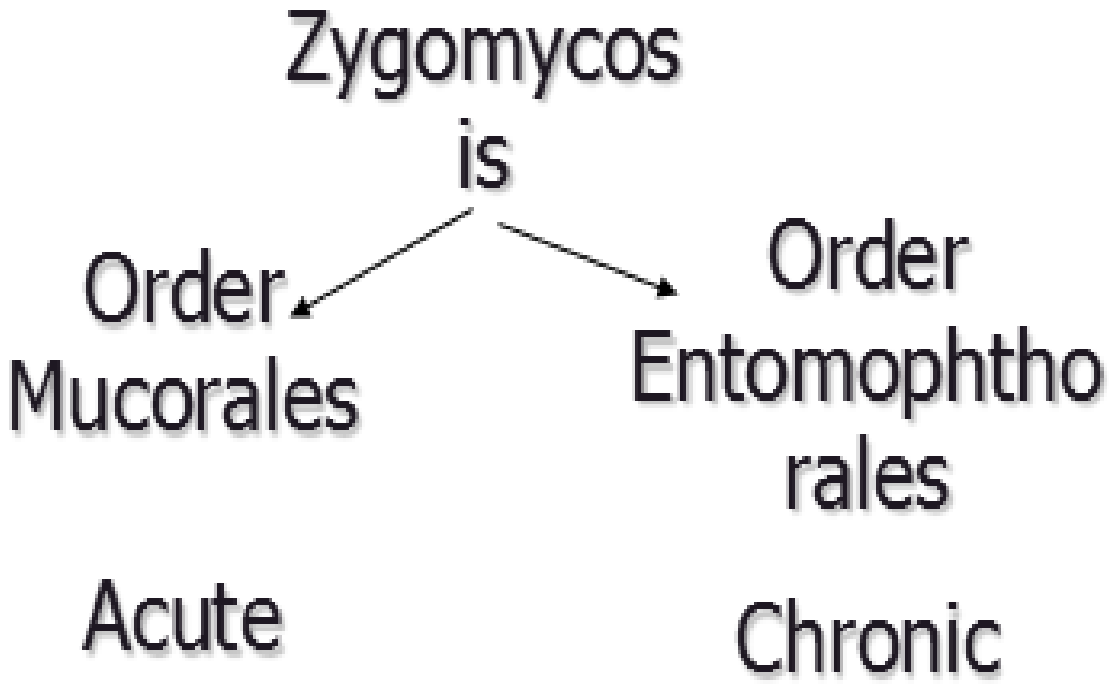

Zygomycosis
Order Mucorales
Order Entomophthorales
Acute
Chronic

- **Classification based on molecular phylogenetic studies of rRNA, tef1, and rpb1, has abolished the class Zygomycetes and instead distributes fungi previously in the phylum Zygomycota into the phylum Glomeromycota and four subphyla, including Mucoromycotina, Kickxellomycotina, Zoopagomycotina, and Entomophthoromycotina.**

Table 1 New taxonomy of fungi previously belonging to the phylum *Zygomycota* (Adapted from [1])

Rank	Taxon
Phylum	Glomeromycota
Subphylum	Mucoromycotina
Order	Mucorales, Endogonales, Mortierellales
Subphylum	Entomophthoromycotina
Order	Entomophthorales
Subphylum	Zoopagomycotina
Order	Zoopagales
Subphylum	Kickxellomycotina
Order	Kickxellales, Dimargaritales, Harpellales, Asellariales

Mycology

- Fungi of the order Mucorales are classified into six different families based on morphologic analysis of the fungi, including the presence and location of rhizoids, the presence of apophyses, and the morphology of the columellae. Other taxonomically relevant features include carbohydrate assimilation and the maximal growth temperature.

Zygomycosis (Mucorales). Mucorales infections Definition:

•Angiotropic (blood vessel-invading)

•The most common genera causing disease are:
- *Rhizopus*
- *Rhizomucor*
- *Mucor*
- *Absidia*

•Fast growing *non-septate* molds

Epidemiology

- Agents of mucormycosis are ubiquitous and thermotolerant organisms that usually grow in decaying matter, including bread, vegetables, fruits, and seeds. They can also be recovered from soil, compost piles, and animal excreta. Most of the Mucorales can grow and sporulate abundantly on any carbohydrate-containing source.

- Abundant growth with sporulation is usually seen in culture media within 2–5 days. The spores are easily airborne, and Mucorales are readily recovered as contaminants in laboratory cultures.
- Indeed, the ability of *R. microsporus* var. *rhizopodiformis* to grow on nonsterile wooden sticks used for culturing stool samples from immunocompromised patients has led to misdiagnosis of patients with gastrointestinal mucormycosis.

Risk factors

- The disease is associated with:
  - Diabetic ketoacidosis
  - Malnourished children
  - Severely burned patients
- It is also seen in patients with
  - Leukemia
  - Lymphoma
  - AIDS
  - In patients using corticosteroids

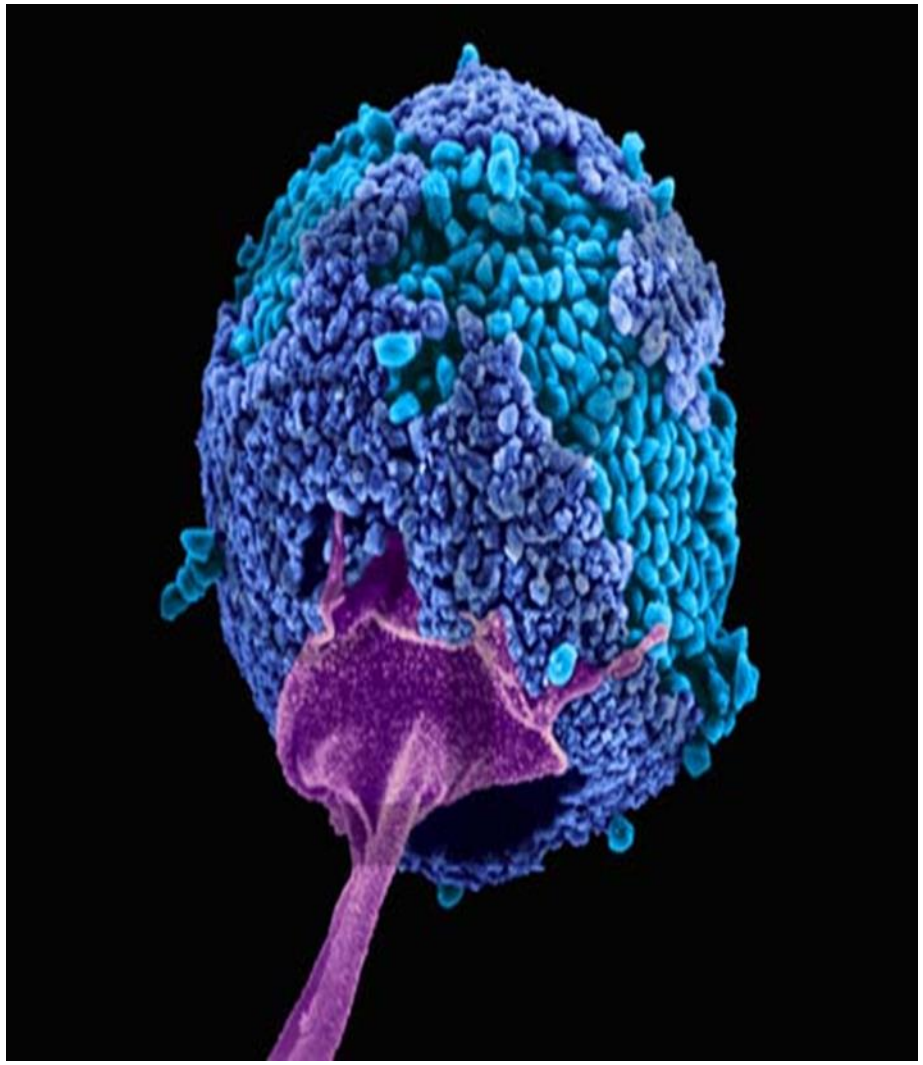
- Other routes of infection include direct implantation into skin, causing local cutaneous infection, and ingestion of contaminated food, which leads to gastrointestinal mucormycosis in highly immunocompromised patients and premature neonates.

Geographical distribution & normal habitat

- World-wide
- Soil and decomposing organic matter
- Found in outdoor and indoor air
- Food and dust

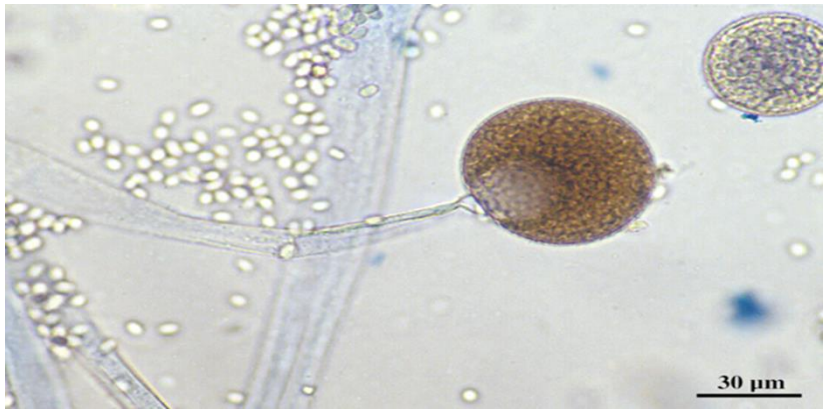
Morphology

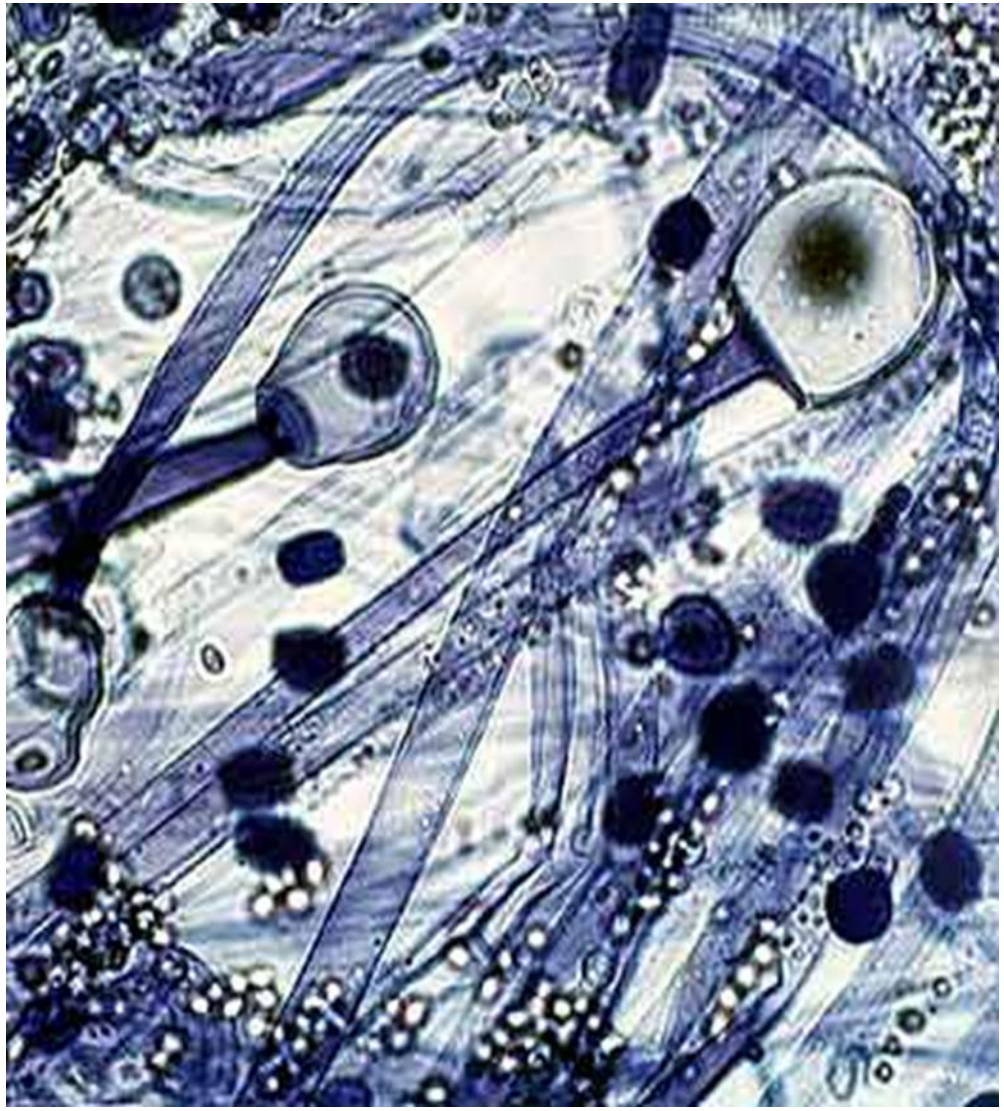
- Majority are with Broad aseptate mycelium with many number of asexual spores inside a sporangium which develops at the end of the aerial hyphae



Mucor

- Microscopy
- Non septate hyphae
- Having branched sporangiophores
- with sporangium at terminal ends





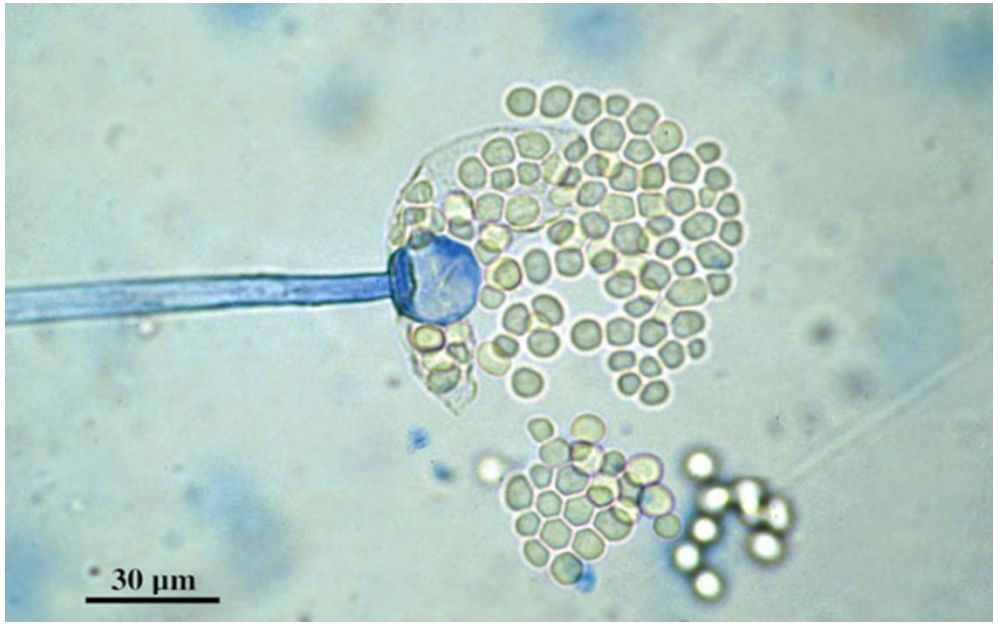
Rhizopus

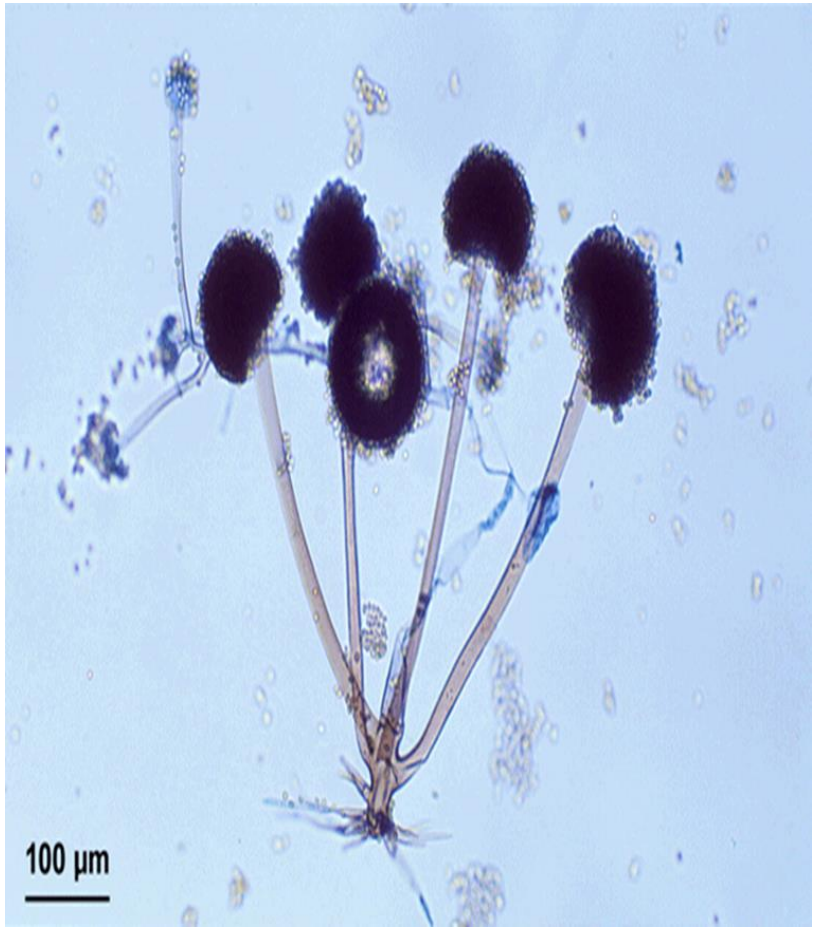
- Microscopy

  Shows non septate hyphae

  Sporangiophores in groups

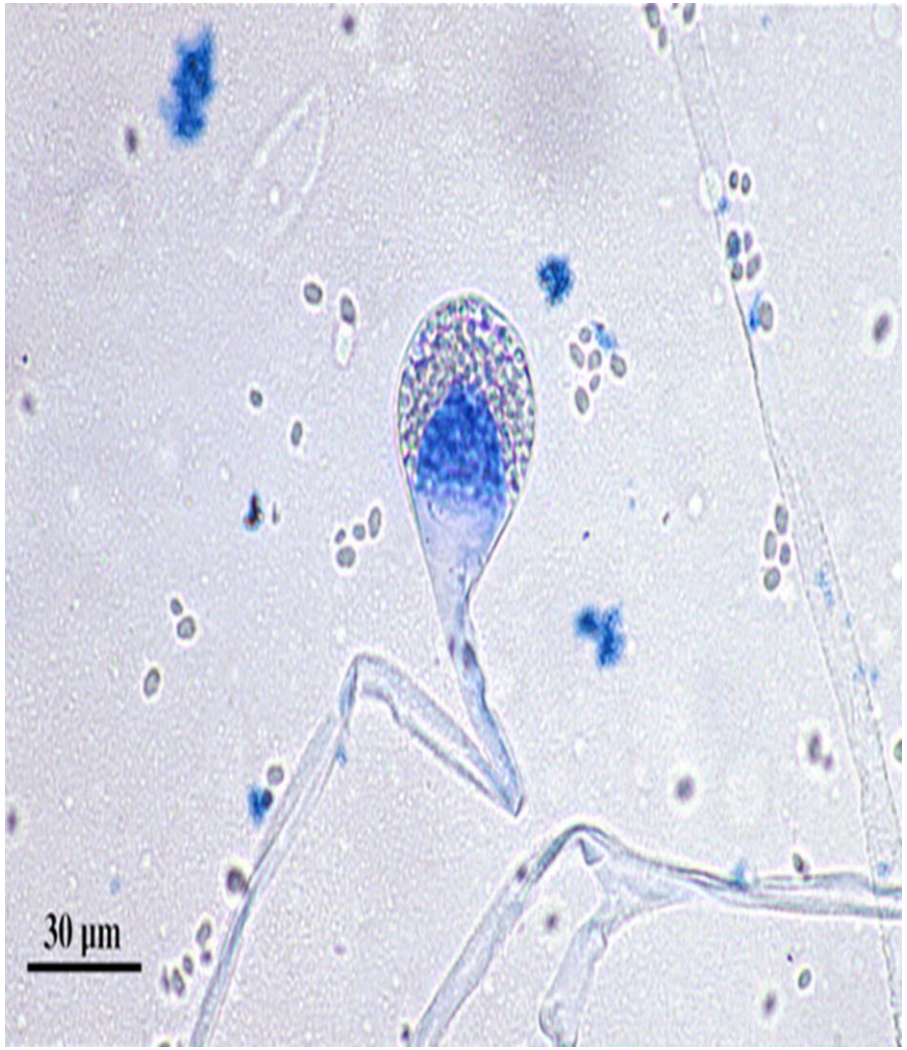
  they are above the Rhizoids





Absidia

a typical pyriform shaped sporangium with a characteristic conical shaped columella and pronounced apophysis. i.e. a swelling of the sporangiophore below the columella.



Pathogenesis

Host Defenses

- The pathogenesis of mucormycosis has been investigated in both in vitro and in animal models. Animal models have included mice or rabbits with mild diabetic ketoacidosis induced by streptozotocin or alloxan, cortisone-treated mice, neutropenic mice, and deferoxamine-treated animals.Inhalation of Mucorales spores by immunocompetent animals does not result in the development of mucormycosis.
- In contrast, when the animals are immunosuppressed by corticosteroids or by induction of diabetes, the animals die from progressive pulmonary and hematogenously disseminated infection.
- Bronchoalveolar macrophages harvested from lungs of immunocompetent mice are able to ingest and inhibit germination of *R. oryzae* spores, preventing progression of the disease.

- In contrast, bronchoalveolar macrophages of immunosuppressed mice are unable to prevent germination of the spores in vitro or after intranasal infection.
- Severely neutropenic patients are at increased risk for developing mucormycosis. In contrast, patients with the acquired immunodeficiency syndrome (AIDS) do not seem to be at increased risk for developing mucormycosis.
- These findings suggest that neutrophils, but not necessarily T lymphocytes, are critical for inhibiting fungal spore proliferation. Recruitment of neutrophils to sites of infection occurs in response to fungal constituents and activation of the alternative complement pathway.
- Both mononuclear and polymorphonuclear phagocytes of normal hosts kill Mucorales by generating oxidative metabolites and the cationic peptides, defensins.

- In the presence of hyperglycemia and low pH, as found in patients with diabetic ketoacidosis, phagocytes are dysfunctional and have impaired chemotaxis and defective intracellular killing by both oxidative and nonoxidative mechanisms.

The Role of Iron in Pathogenesis

- It has recently been discovered that a specific factor that uniquely predisposes patients in diabetic ketoacidosis to mucormycosis is the level of available unbound iron in serum.
- Iron is required by virtually all microbial pathogens for growth and virulence. In mammalian hosts, very little serum iron is available to microorganisms because it is highly bound to host carrier proteins, such as transferrin.
- Sequestration of serum iron is a major host defense mechanism against microbes in general and Mucorales in particular, because *Rhizopus* grows poorly in normal serum unless exogenous iron is added.
- Furthermore, the bacterial siderophore, deferoxamine, predisposes patients to *Rhizopus* infection by acting as a xenosiderophore, which supplies previously unavailable iron to the fungus.

- The mechanism by which *Rhizopus* obtains iron from the iron-deferoxamine complex involves binding of this complex to the mold, followed by active reduction of the iron by the fungus, resulting in release of iron from deferoxamine and subsequent transport of the reduced iron intracellularly. This transport is likely mediated by iron permeases. Administration of deferoxamine worsens survival of guinea pigs infected with *Rhizopus* but not *Candida albicans*. Additionally, in vitro studies of radiolabeled iron uptake from deferoxamine in serum show that *Rhizopus* is able to incorporate eightfold and 40-fold more iron than is *Aspergillus fumigatus* and *C. albicans,* respectively.

- patients with diabetic ketoacidosis are at high risk of developing rhinocerebral mucormycosis. These patients have elevated levels of available iron in their serum, and such serum supports growth of *R. oryzae* at acidic pH (7.3–6.88) but not at alkaline pH (7.7– 8.38).
- adding exogenous iron to sera allowed *R. oryzae* to grow profusely at acidic conditions but not at (pH ≥ 7.4).
- Recent animal data showed that mice with diabetic ketoacidosis were protected from *Rhizopus* infection by administering iron chelators, such as deferiprone and deferasirox that are not utilized by *Mucorales* as xenosiderophores.

- Fungi can obtain iron from the host by using low-molecular- weight iron chelators (siderophores) or high-affinity iron permeases [39, 45]. Because the siderophores of *Rhizopus* species are very inefficient at obtaining iron from serum.
- The high-affinity iron permeases are able to transport serum iron intracellularly, and are therefore likely to be critical for the survival of the organism in susceptible hosts.
- high-affinity iron permease (*rFTR1*) is expressed by *R. oryzae* during murine infection. Inhibition of *rFTR1* gene expression by RNA-i or reduction of *rFTR1* copy number by gene disruption reduces the virulence of the fungus in animal models of mucormycosis.

- A third mechanism by which fungi can obtain iron from the host is through utilization of hemin. The *Rhizopus* genome project revealed two homologues (RO3G_07326 and RO3G_13316) of the heme oxygenase (CaHMX1). These two *R. oryzae* homologues may enable *R. oryzae* to obtain iron from host hemoglobin, and might explain the angioinvasive nature of *R. oryzae*.

Mucorales–Endothelial Cell Interactions

- mucormycosis is the virtually uniform presence of extensive angioinvasion with resultant vessel thrombosis and tissue necrosis. This angioinvasion likely contributes to the capacity of the organism to hematogenously disseminate to other target organs. Therefore, damage of and penetration through endothelial cells or the extracellular matrix proteins lining blood vessels is likely a critical step in *R. oryzae*'s pathogenetic strategy.
- An earlier study showed that *R. oryzae* can adhere to the extracellular matrix laminin and type IV collagen.

- More recently, it has been shown that *R. oryzae* spores and hyphae are able to damage human umbilical vein endothelial cells in vitro.
- It has also been shown that injury requires adherence of the fungus to endothelial cells followed by invasion into the cells. Adherence to endothelial cells is believed to be mediated by a specific receptor since it was found to be saturable.
- Glucose-regulated protein (GRP78) acts as a receptor which mediates penetration through and damage of endothelial cells by Mucorales. GRP78 (also known as BiP/HSPA5) is a member of the HSP70 protein family, and some of it is located on the cell surface. It is a key regulator of the unfolded protein response (UPR).

- Elevated concentrations of glucose and iron consistent with those noted during diabetic ketoacidosis enhanced surface GRP78 expression and resulting penetration through and damage of endothelial cells by Mucorales in a receptor-dependent manner.

Mycotoxins

- *Rhizopus* species are also known for their ability to produce mycotoxins, such as the macrocyclic polyketide metabolite, rhizoxin, as well as the cyclic peptides, rhizonins A and B.
- A study demonstrated that the mycotoxin rhizoxin is not biosynthesized by *Rhizopus* itself, but rather by an intracellular, symbiotic bacterium of the genus *Burkholderia*.
- This bacterium is sensitive to antibiotics belonging to the fluoroquinolone family. For example, rhizoxin production is completely abrogated when *Rhizopus* is grown in media containing 40 mg/mL of ciprofloxacin. Rhizoxin has long been known to be crucial to the plant pathogenic strategy of *Rhizopus,* but it does not appear to have a substantive role in causing mammalian disease.

- Other putative virulence factors include the ability of *Rhizopus* to secrete lytic enzymes, including aspartic proteinases. Additionally, *Rhizopus* species have an active ketone reductase system which may be an additional virulence factor that functions by enhancing growth in the acidic and glucose-rich environment seen in ketoacidotic states.

Clinical Manifestations

- Based on clinical presentation and the involvement of a particular anatomic site, mucormycosis can be divided into at least five categories: (1) rhinocerebral, (2) pulmonary, (3) cutaneous, (4) gastrointestinal, and (5) disseminated.

Rhinocerebral

- Rhinocerebral mucormycosis is the most common form of the disease, representing between one third to one half of all cases.
- About 70% of cases of rhinocerebral (occasionally referred to as craniofacial) mucormycosis are found in diabetic patients with ketoacidosis.
- Rhinocerebral mucormycosis is increasingly being encountered in patients receiving high doses of corticosteroids, such as those with rheumatologic disorders, and those who have received an organ transplant.
- Causes extensive Cellulitis, and tissue destruction.

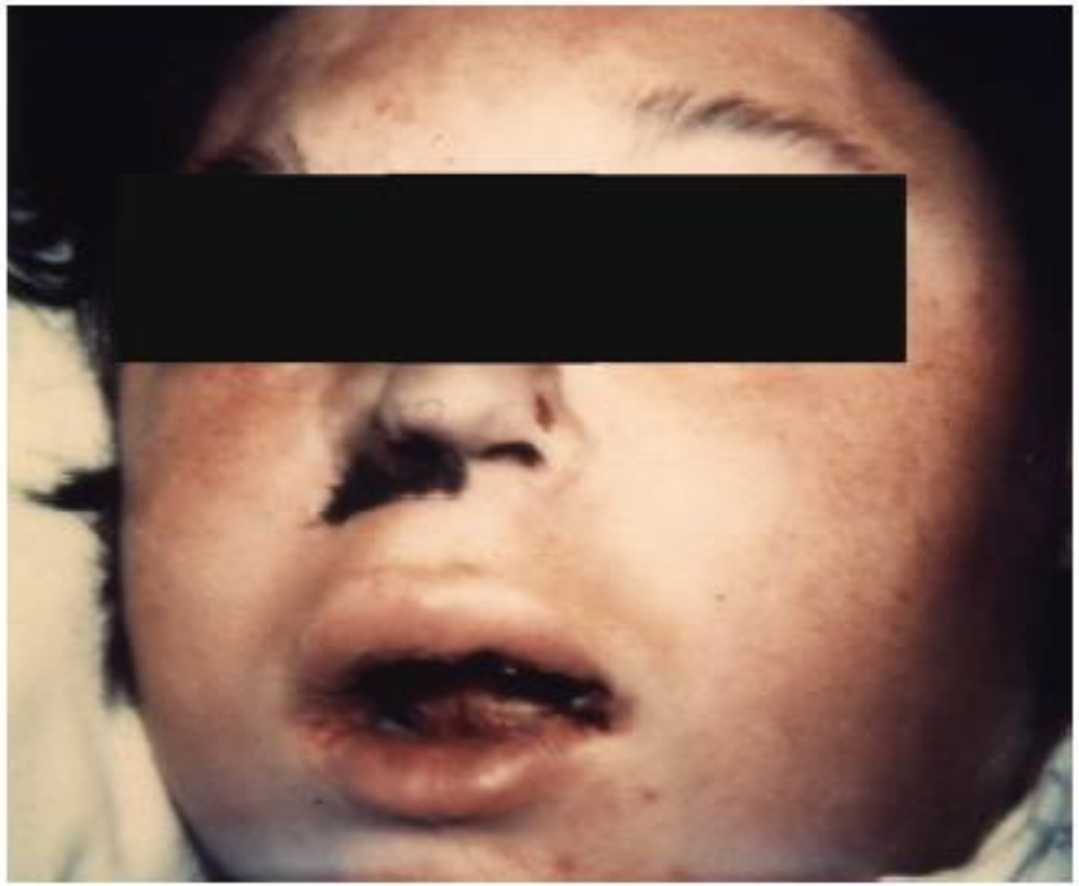


Fig. 2 Rhinocerebral mucormycosis in a pregnant woman who had diabetic ketoacidosis. Note bilateral swelling and infarction of skin of nose and infranasal tissue. There was also gangrenous ulceration of the palate

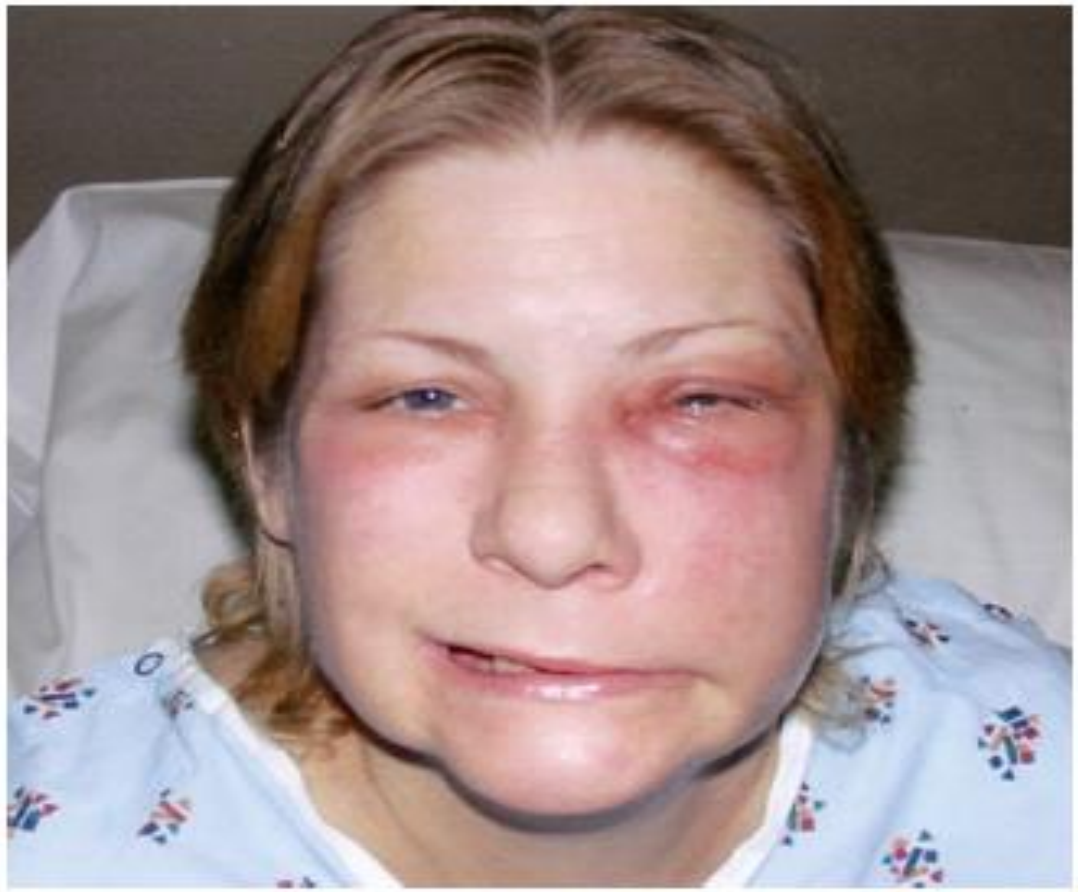


Fig. 3 Rhinocerebral mucormycosis in a patient who had diabetic ketoacidosis. Note swelling, erythema, proptosis, ptosis, and peripheral left facial nerve palsy

Pulmonary

- PULMONARY MUCORMYCOSIS

- Seen most commonly in –neutropenia,pateints on chemotherpy,leukemia.
- Dysponea ,cough& chest pain &fever.
- Radiologicaly-consolidation,isolated masses,cavitaion,wedge shaped infarcts.
- CT scan best method to detect the extent.

Cutaneous

- **Cutaneous mucormycosis can occur following:**
- 1-traumatic implantation of soil or plant material, such as occurs after motor vehicle accidents.
- 2-In diabetic or immunocompromised patients,
- 3-cutaneous lesions may arise at an insulin injection or catheter insertion site.
- 4- A large epidemic of cutaneous mucormycosis was reported in patients who had contaminated surgical dressings applied to their skin.
- 5- Cutaneous mucormycosis may also occur in burn patients.

- Primary infection produces an acute inflammatory response with purulence, abscess formation, tissue swelling, and necrosis.
- The lesions may appear red and indurated, and often progress to black eschars.
- The necrotic tissue may slough and produce large ulcers. Primary cutaneous disease, which may be polymicrobial, is usually rapidly progressive even in the face of appropriate debridement and medical treatment.

- Occasionally, aerial mycelia may be visible on the surface of the cutaneous lesion.
- This form of cutaneous disease can be very invasive locally, and penetrate from the cutaneous and subcutaneous tissues into the adjacent fat, muscle, fascia, and bone.
- Cutaneous and subcutaneous disease may lead to necrotizing fasciitis, which has a mortality rate approaching 80%.
- Secondary vascular invasion may also lead to hematogenously disseminated infection of the deep organs.
- When cutaneous mucormycosis results from hematogenously disseminated infection, the lesion typically begins as an erythematous, indurated, and painful cellulitis, progressing to an ulcerative lesion covered by a black eschar.

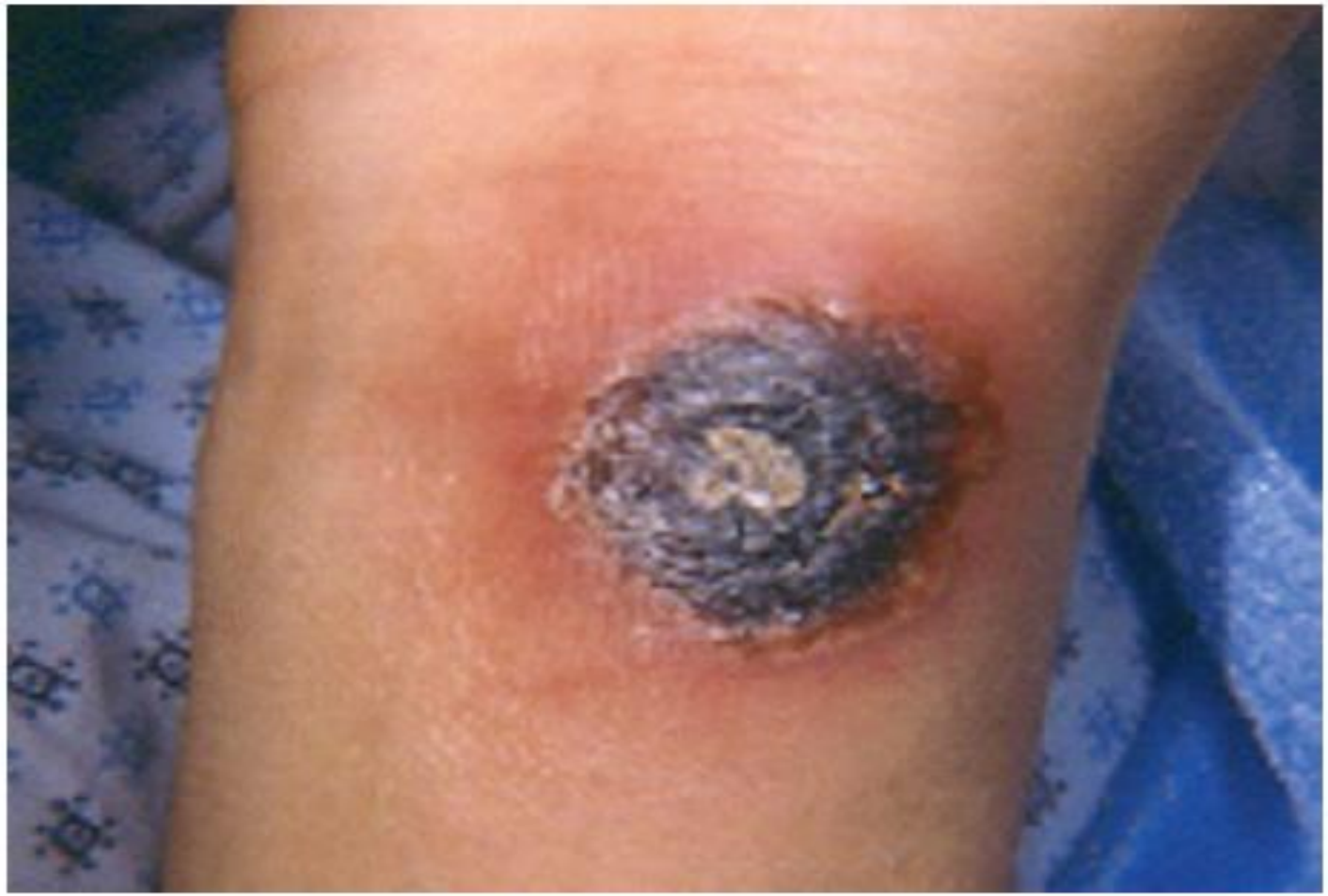


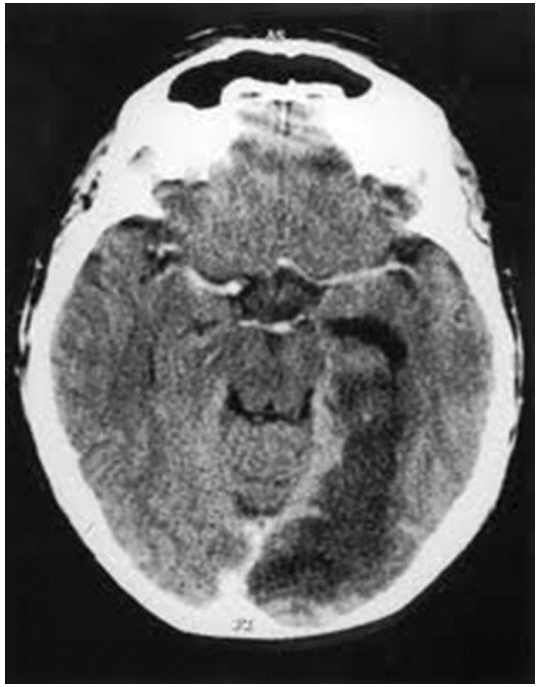
Fig. 4 Cutaneous mucormycosis in a patient who had acute leukemia. Note the black eschar with surrounding erythema. The lesion was quite painful (Courtesy of Dr. Dimitrios Kontoyiannis)

Gastrointestinal

- Rare,,occurs in extremaly malnourished, children.
- Stomach,colon&ileum are most commonly involved.
- Abdominal pain, nausea vomiting, ,,,may presnt as intra abdominal abscess, or perforation of the viscus. needs biopsy.
- prognosis very poor.

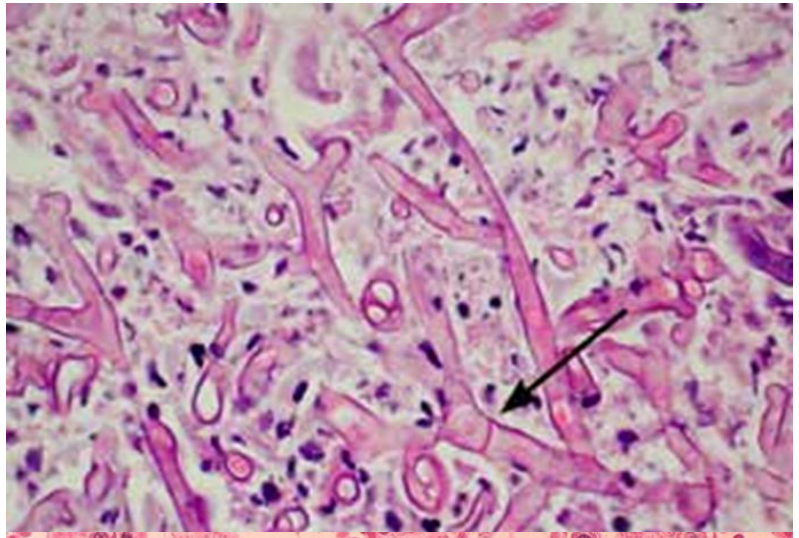
Disseminated

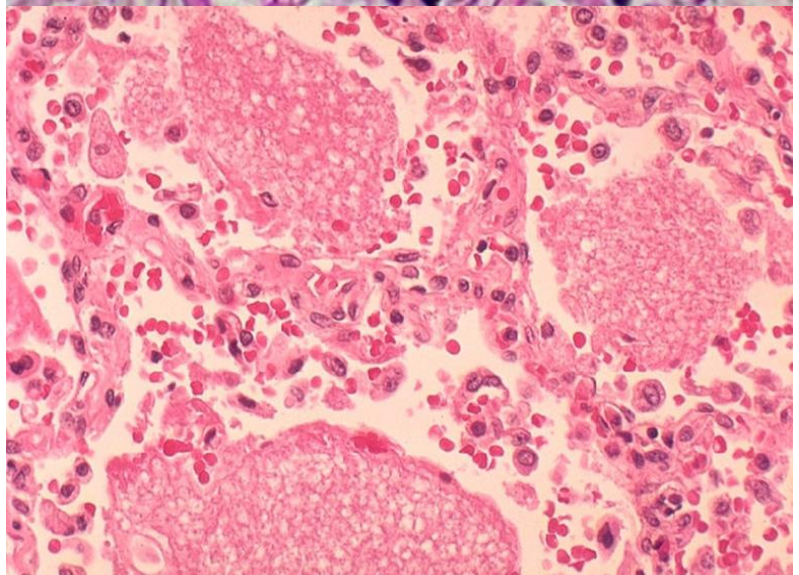
- Heamatogenously
- Pulmonary mucormycosis has highest incidence of dissemination.
- Most common site of dissemination-brain ,,,spleen ,heart,skin, and other organs.
- Brain -100%,others->90%



Laboratory Diagnosis

- Histopathology more reliable than culturing
- A certain Diagnosis needs Biopsy
- Nasal discharges Sputum, rarely contain many fungal elements.
- Histological sections Contain non septate hyphae in thromboses vessels or sinuses surrounded by leukocytes or giant cells.

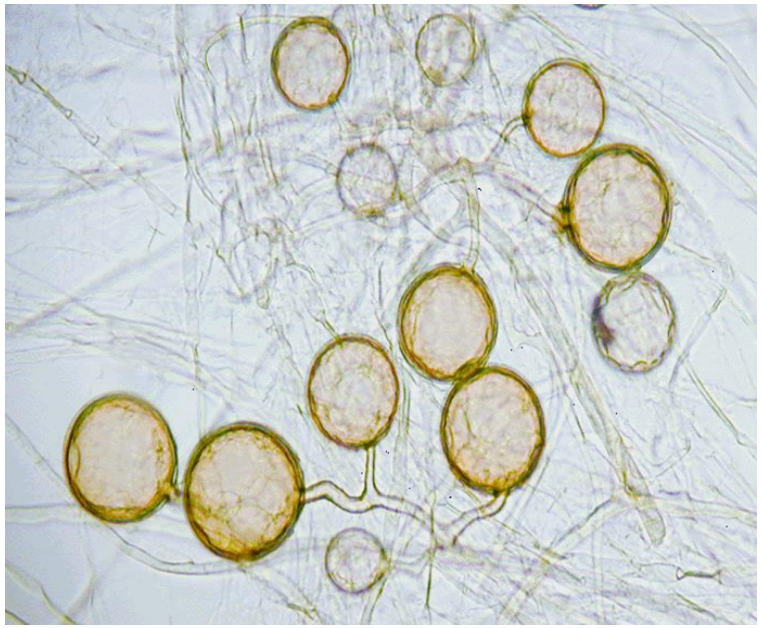


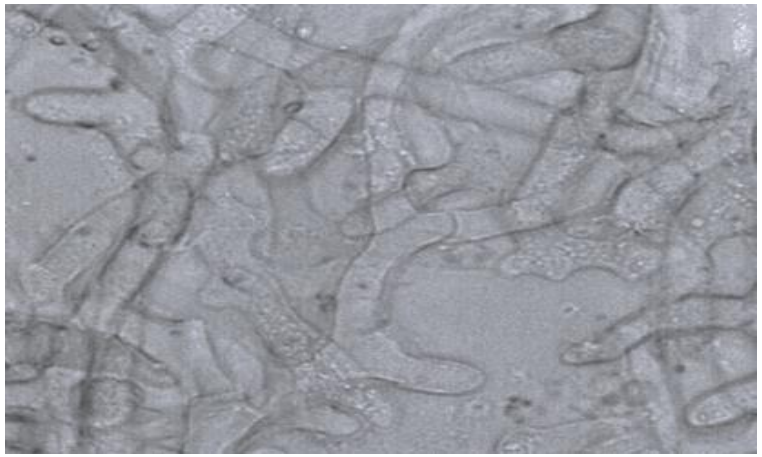


Microscopy

1. **Direct examination** :

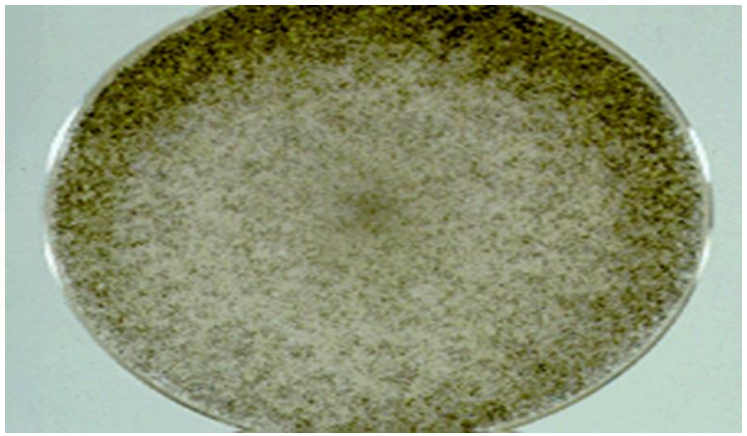
- A rapid diagnosis is critical.
- Fungal elements are usually not numerous in discharges.
- Scrapings from the upper turbinates, aspirated material from sinuses, sputum in pulmonary disease, and biopsy material mounted in 10% KOH typically contain thick-walled, refractile hyphae 6-15 um in diameter.
- Swollen cells (up to 50 um) and distorted hyphae may be present.

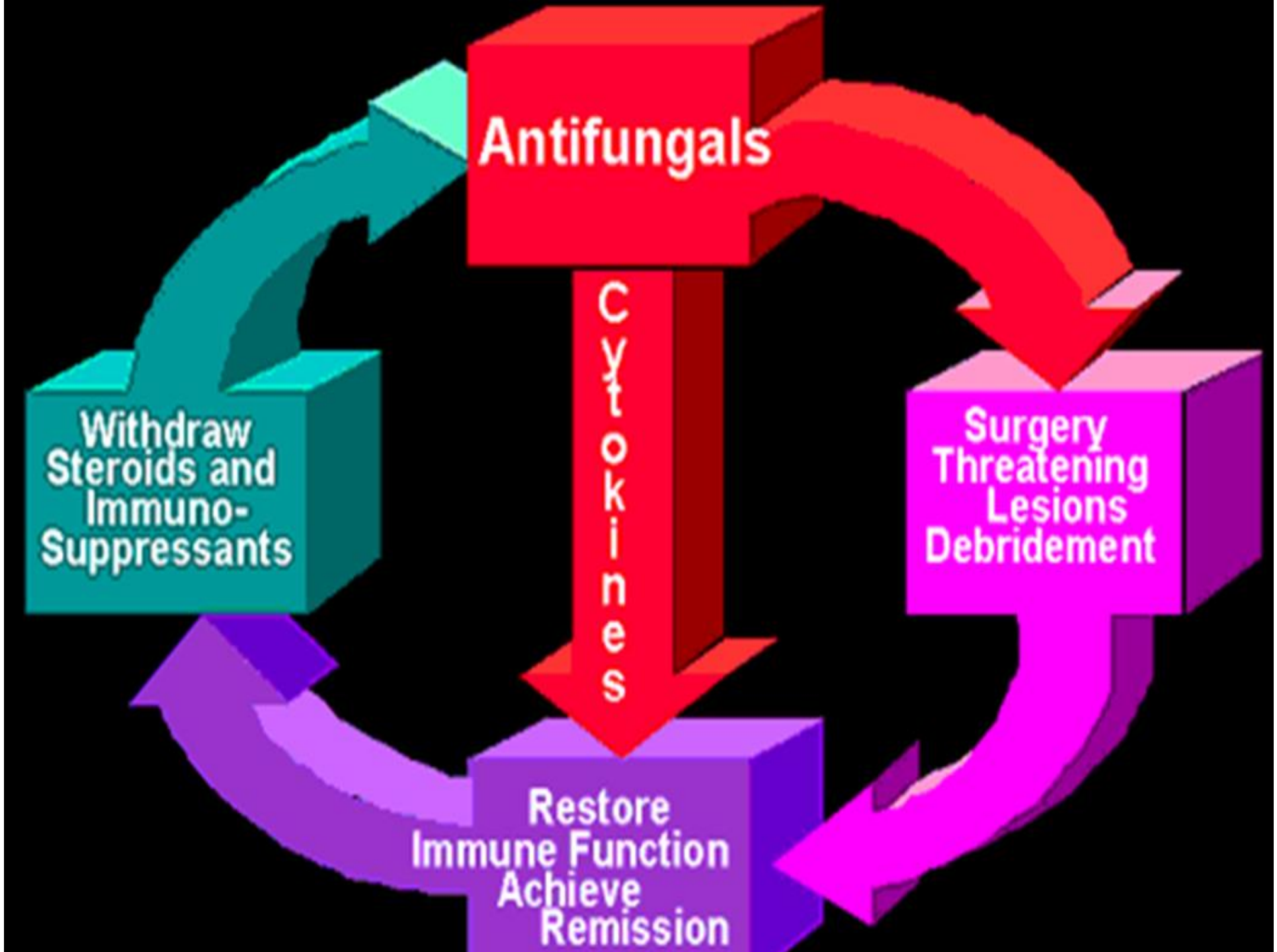




Laboratory 2
Isolation

- Inoculate the clinical material onto Sabouraud dextrose agar and incubate at 30°C.
- A medium containing cycloheximide is not used because these fungi are sensitive to cycloheximide.
- Sterile bread in a test tube may recover Zygomycetes when other media fail.
- A noninoculated tube of sterile bread is necessary for quality control, since Zygomycetes are commonly associated with bread.




Antifungals
Cytokines
Surgery
Threatening
Lesions
Debridement
Restore
Immune Function
Achieve
Remission
Withdraw
Steroids and
Immuno-
Suppressants

Antifungal agents against zygomycetes

Table 4. Susceptibilities of zygomycetes to various antifungal agents*

Organism	Antifungal	MIC_{90} (mcg/mL)	Percent inhibited at MIC ≤1 mcg/mL
Rhizopus species	Posaconazole	1–4	40
	Voriconazole	1–8+	40
	Itraconazole	1–8+	20
	Amphotericin B	0.5–1	100
	Caspofungin	>8	0
Mucor species	Posaconazole	0.5–8+	67
	Voriconazole	1–8+	33
	Itraconazole	2–8+	0
	Amphotericin B	0.5–1	100
	Caspofungin	>8	0

- **Amphotericin B** is the drug of choice for treatment of zygomycosis.

- Duration of therapy is not clearly defined but a total dose of at least 2g of amphotericin B is administered, although some patients have received up to 4g.