Diagnostic Mycology for Laboratory Professionals
Part Three--Opportunistic Molds

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The presenter states no conflict of interest and has no financial relationship to disclose relevant to the content of this presentation.

OUTLINE

I. Introductory statements

   A. Review of classification
   B. Important general criteria

II. Identification of clinically-significant molds

   A. Macroscopic morphology
   B. Microscopic morphology
   C. Other hints

III. Antifungal susceptibility testing

"D#*%it, Jim,
I'm not a physician."

The Basics

SCOPE OF FUNGI

- At least 100,000 named fungal species
- ~1 million to 10 million unnamed species; 1000 to 1500 new species per year
- Fewer than 500 named species associated with animal or human disease
- Less than 50 are pathogenic in healthy human hosts

Biol. Rev. **73:** 203-266; 1998

PATHOGENICITY OF FUNGI

-- Generally more chronic than acute

-- Generally involves predisposition

Chemotherapy-induced neutropenia	HIV
Organ transplantation	Diabetes
Corticosteroids	Alcoholism
Broad-spectrum antimicrobials	Intravenous drug abuse
Parenteral nutrition	Intensive care population (burns, NICU)
Dialysis	Malignancy
Invasive medical procedures	Other immune deficiency

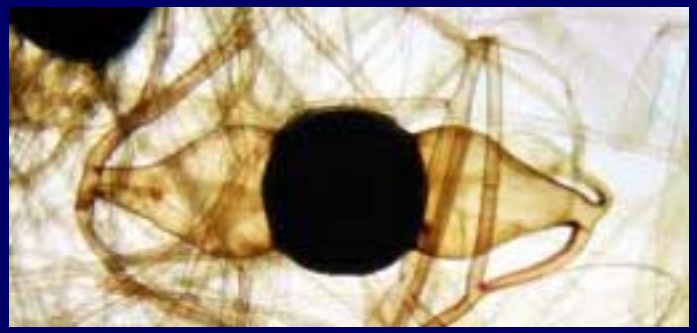
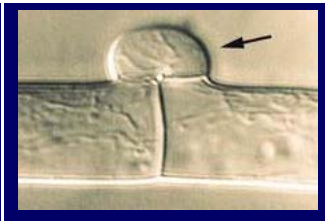
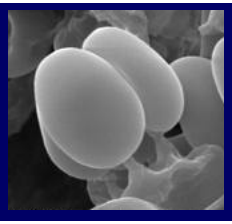
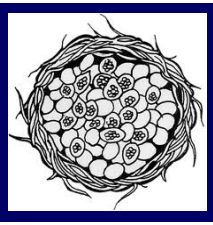
-- Certain infections can be “signal diseases”

CLASSIFYING OPPORTUNISTS

- Taxonomy

Holomorph	
Teleomorph	Anamorph
Sexual reproduction	Asexual reproduction
Fusion of two nuclei into zygote	Mitosis
Perfect Fungi	“Fungi Imperfecti”
Pseudallescheria boydii	*Scedosporium apiospermum*

SEXUAL REPRODUCTION

Subphylum Mucoromycotina	Zygophores meet and fuse (zygosporangium) 
Phylum Basidiomycota	  Clamp connections facilitate basidium
Phylum Ascomycota	   Nuclear division inside ascus (bag)
Phylum Deuteromycota	NO SEXUAL REPRODUCTION OBSERVED

CLASSIFYING OPPORTUNISTS

- Taxonomy
- Cell morphology (conidiogenesis)

Blastic	blastoconidia	annelloconidia
Enlarge, then divide	phialoconidia	poroconidia
Thallic	arthroconidia	aleurioconidia
"Divide", then enlarge		chlamydoconidia

- Mode of entry (implantation; inhalation)

UNIFYING CONCEPTS

Macroscopic observation of colonial growth

Microscopic observation of colonial growth

Growth on selective medium

Rate of growth

Pigmentation

Wild Card

DERMATOPHYTES

- Infrequent mortality
- Tinea (ringworm)
- Immunocompromised host not required
- Some have niche in terms of parasitism

Geophilic	*M. gypseum*
Zoophilic	*M. canis* *T. mentagrophytes*
Anthropophilic	Most

DERMATOPHYTES

- Some have regions of endemicity

M. audouinii	Africa, Haiti
T. violaceum	Middle East, North Africa
T. concentricum	Polynesia Pockets of C. and S. America

Dermatophyte	Nails	Skin	Hair
Microsporum spp.	NO	Yes	Yes
Epidermophyton floccosum	Yes	Yes	NO
Trichophyton spp.	Yes	Yes	Yes

DERMATOPHYTES

Groups	Agents	Cultures	%	Total	%
Anthropophilic	*T. rubrum*	319	(48.7)	501	(76.4)
	T. tonsurans	91	(13.8)		
	T. mentagrophytes	64	(9.7)		
	E. floccosum	27	(4.1)		
Zoophilic	*M. canis*	137	(20.9)	137	(20.9)
Geophilic	*M. gypseum*	17	(2.5)	17	(2.5)

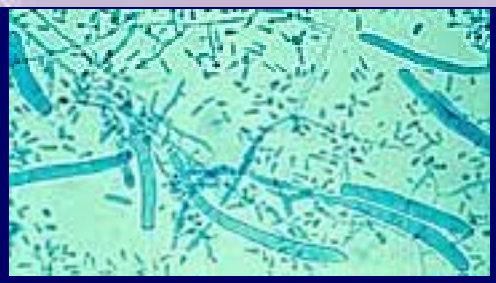
Rev. Inst. Med. trop. S. Paulo
45: 259-263; 2003

Organisms	Number	Percent
Trichophyton tonsurans	359	56
Epidermophyton floccosum	76	11.8
T. mentagrophytes	57	8.9
T. rubrum	53	8.3
T. verrucosum	25	3.9
T. violaceum	21	3.3
M. canis	16	2.5
Malassezia fur fur	21	3.3
Pityrosporum oval	13	2
Total	641	100

Ann. Trop. Med. Pub. Health
3: 53-57; 2010

Trichophyton rubrum

- ~14 days; resistant to cycloheximide
- Diffusible red pigment
- Smooth-walled “pencil” macroconidia (3-8 cells) variable in amount
- Abundant microconidia; tear-shaped (“birds on a wire”)
- Urease-negative after 7 days



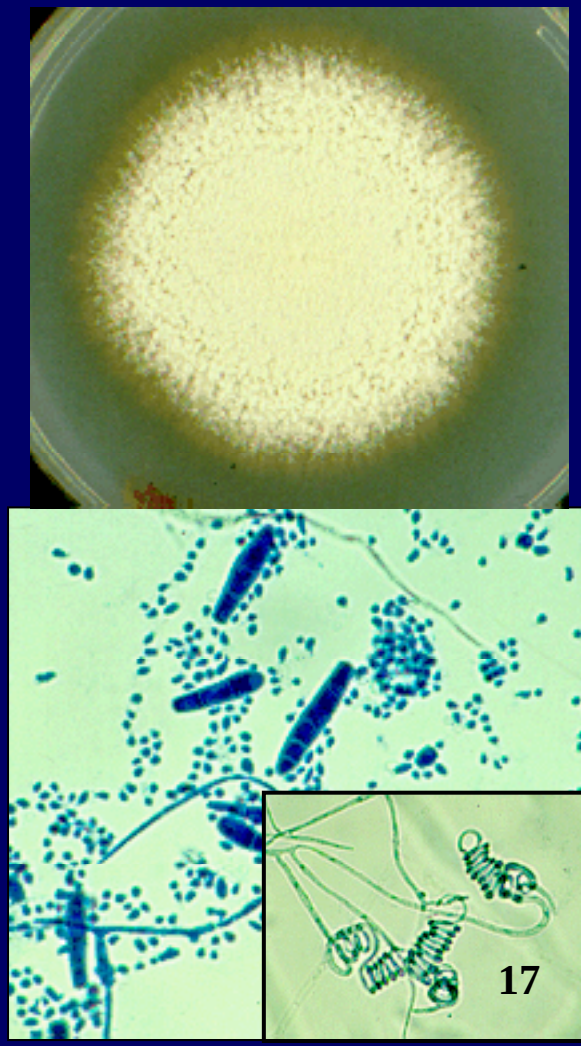
Trichophyton tonsurans

- ~12 days; resistant to cycloheximide; scalp
- Suede surface with folds
- Rare, irregular, thick-walled macroconidia
- Abundant microconidia (tears, balloons, clubs); some elongated
- Urease-positive after 4 days

Trichophyton mentagrophytes

- ~7-10 days; resistant to cycloheximide; foot

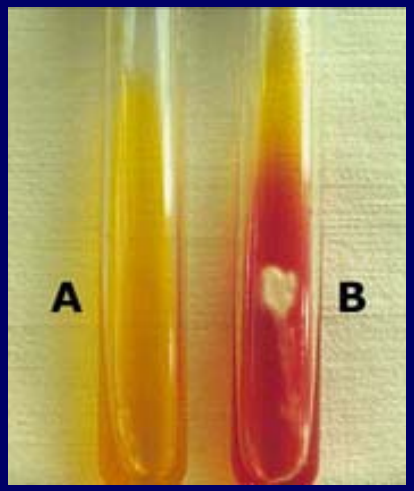
Fluffy, white	Variable-pigment, granular
Rare macroconidia	Cigar-shaped, smooth, thin-walled (1-6 cells); narrow attachment to hyphae
Small microconidia; tear-shaped (resembling *T. rubrum*)	Very round microconidia; clustered on branched conidiophores



- Spiral hyphae
- Urease-positive after 4 days

Trichophyton AGARS

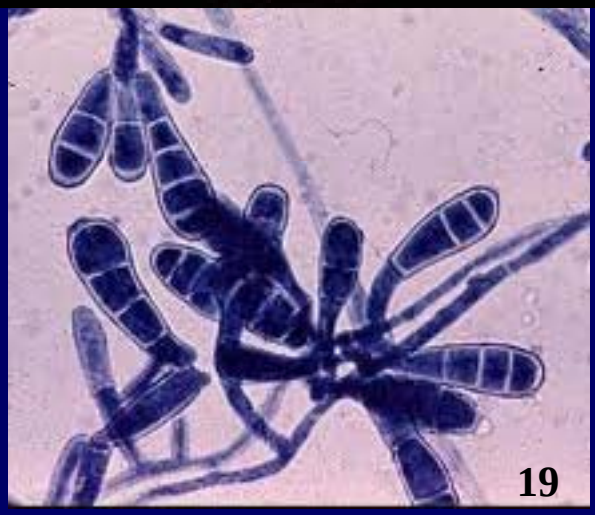
- Homogenous suspension of mycelial growth
- Room temperature; 2 weeks



Selected *Trichophyton* spp.	Growth in Presence of:			
	Casein		Ammonium nitrate	
	Base	+ thiamine	Base	+ histidine
T. rubrum	4+	4+	3+	4+
T. tonsurans	1+	4+	1+	1+
T. mentagrophytes	4+	4+	4+	2+

Epidermophyton floccosum

- ~10 days; resistant to cycloheximide; jock
- Starts velvety and khaki; becomes fluffy white
- Smooth, thin- or thick-walled macroconidia; rounded ends; single or characteristic clusters
- No microconidia
- Urease-positive after 7 days



Microsporum canis

- ~6-10 days; resistant to cycloheximide; kids
- Cottony, wooly; lemon periphery closely-spaced grooves
- Rough, thick-walled, spindle-shaped macroconidia; tapers to knob-like ends (6-15 cells)
- Rare, single microconidia
- Urease-positive after 7 days

Microsporum gypseum

- ~6 days; resistant to cycloheximide; kids
- Cinnamon brown to buff; granular (sporulates heavily)
- Very abundant macroconidia; thin-walled with rounded tips (4-6 cells)
- Rare, single microconidia
- Urease-positive after 7 days

Pictures

DEMATIACEOUS OPPORTUNISTS

- Soil, plant, moist organics (some air)
- Some tropical; some temperate
- Immunocompromised host not required
- Spectrum of disease

  Eumycotic mycetoma
  Chromoblastomycosis
  Phaeohyphomycosis
  Chronic sinusitis (portal for CNS disease)
  Rare systemic disease

Eumycotic mycetoma
with *Exophiala* etiology

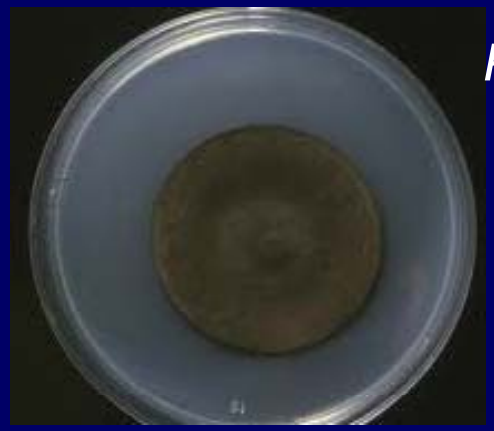
Chromoblastomycosis
with *Phialophora* etiology

Phaeohyphomycosis
with *Alternaria* etiology

Fonsecaea spp. AND OTHERS

- Most common worldwide cause of chromoblastomycosis
- Maturity in ~14-28 days
- Colony surface dark green, black, or gray; reverse is black
- Conidia (phores), hila, vase-shaped phialides, collarettes, denticles

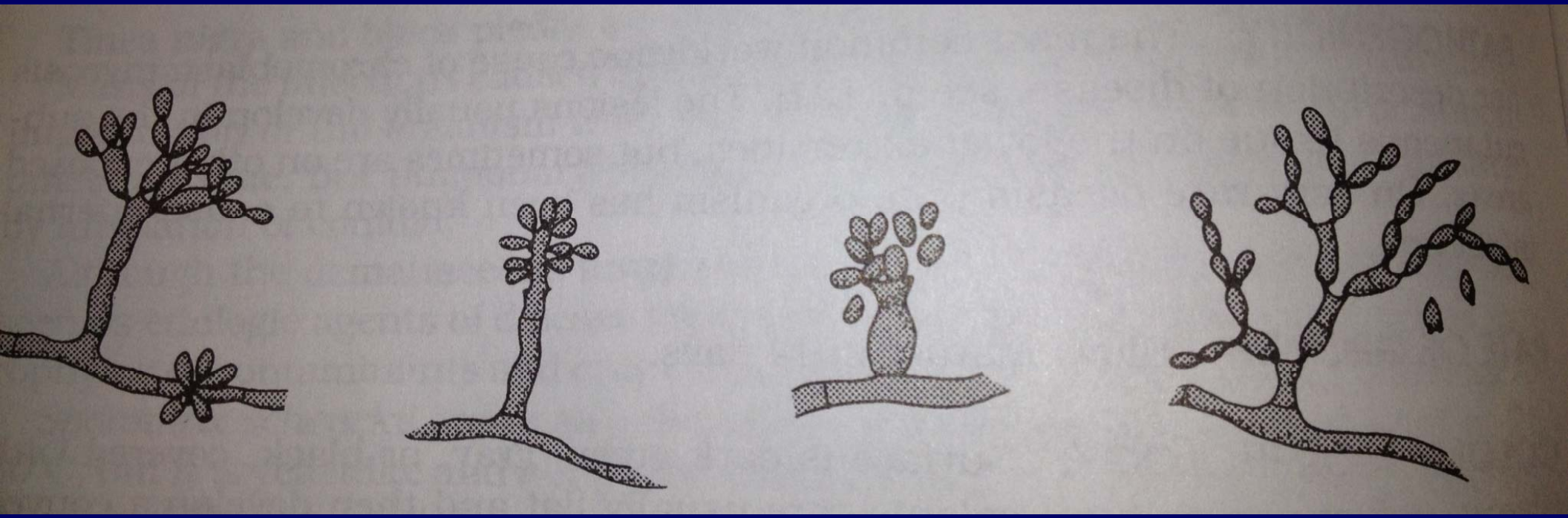
Fonsecaea pedrosoi



Rhinocladiella spp.

Phialophora spp.

Fonsecaea spp. AND OTHERS



Fonsecaea-type conidiation

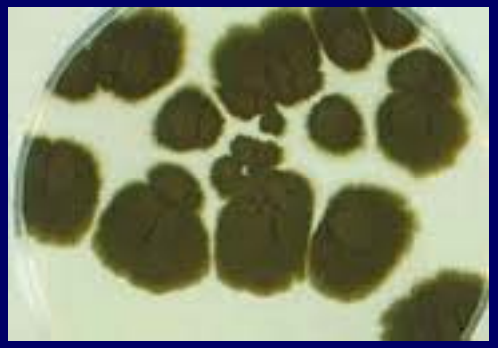
Rhinocladiella-type conidiation

Phialophora-type conidiation

Cladosporium-type conidiation

Cladosporium spp., *Cladophialophora*

- Most common worldwide cause of chromoblastomycosis
- Maturity in ~14-28 days
- Colony surface dark green, black, or gray; reverse is black
- Conidia (phores), hila, vase-shaped phialides, collarettes, denticles

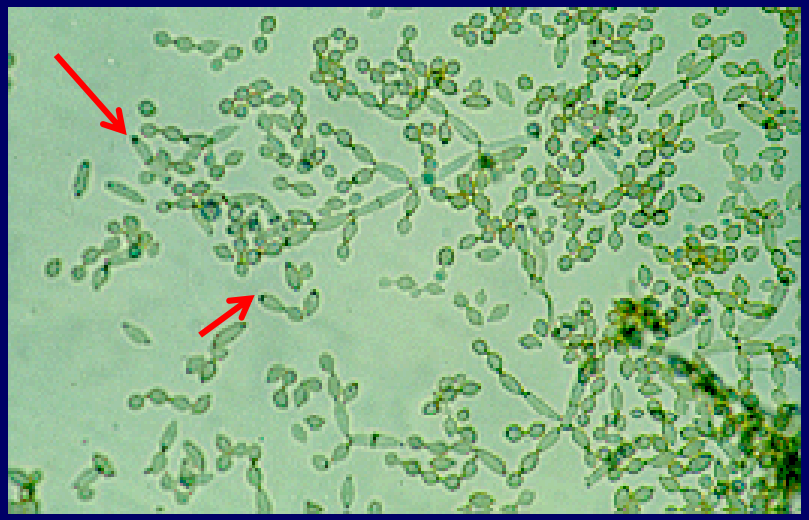


Cladosporium spp.

Cladophialophora carrionii
(formerly *Xylohypha*)

Cladophialophora bantiana
(formerly *Xylohypha*)

Cladosporium spp., *Cladophialophora*



Cladosporium spp.

Cladophialophora bantiana

Cladophialophora carrionii

Dematiaceous Mold	Distinct conidiophores	Hila on conidia	Conidial chain length	Conidial chain branching	Gelatin hydrolysis	Growth in 15% NaCl	Max growth ° C
Cladosporium spp.	Yes	Yes	Short	Frequent	Positive	Positive	<37
C. carrionii	Variable	Yes	Medium	Moderate	Negative	Negative	35-37
C. bantiana	No	No	Long	Sparse	Negative	Negative	42-43

D. H. Larone, Medically Important Fungi, fourth ed.

Alternaria spp.

- Typically contaminant; role in phaeohyphomycosis, allergy
- Maturity in ~5 days
- Colony surface becomes greenish black or brown with light border; reverse is black
- Drumstick macroconidia with longitudinal, transverse septations; poroconidiation (chains)

Inhalation

ASPERGILLOSIS

- Nasoorbital
- Endocardial
- Cutaneous
- Disseminated
- Central nervous system disease
- Pulmonary

  Allergic bronchopulmonary aspergillosis
  Aspergilloma (fungus ball)
  Invasive pulmonary aspergillosis

24 MEDICAL CENTERS; n = 1477

What does your (lab) positive culture result mean???

Aspergillus species	No. (%) of positive culture results, according to clinical condition					
	Invasive disease (n = 256)	Chronic necrotizing aspergillosis (n = 41)	Aspergilloma (n = 83)	ABPA (n = 87)	Colonization (n = 735)	Contamination (n = 275)
A. flavus	41 (16)	1 (2)	2 (2)	5 (6)	66 (9)	29 (10)
A. fumigatus	171 (67)	33 (80)	57 (69)	80 (92)	465 (63)	102 (37)
A. nidulans	2 (1)	0 (0)	0 (0)	0 (0)	5 (1)	2 (1)
A. niger	14 (5)	4 (10)	11 (13)	0 (0)	101 (14)	66 (24)
A. terreus	8 (3)	0 (0)	0 (0)	0 (0)	8 (1)	1 (1)
Other	2 (1)	3 (8)	4 (5)	0 (0)	28 (4)	17 (6)
Not identified	18 (7)	0 (0)	9 (11)	2 (2)	62 (8)	58 (21)

NOTE. ABPA, allergic bronchopulmonary aspergillosis.

Clin. Infect. Dis. **33:** 1824-1833; 2001

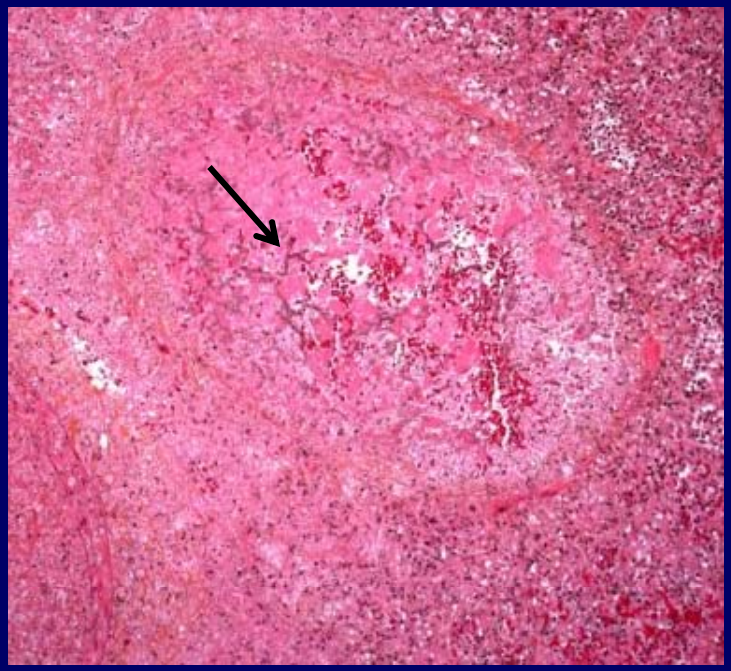
UNDERLYING RISK AND OUTCOME

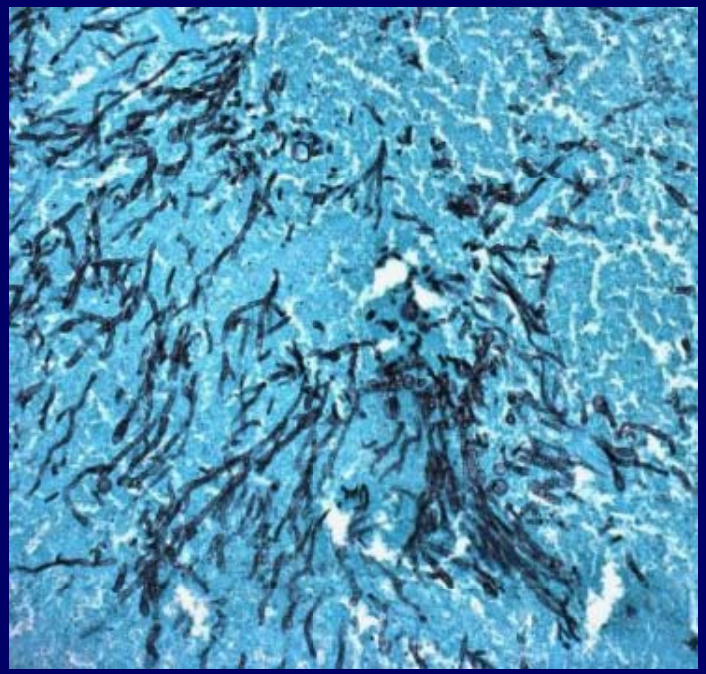
Group, characteristic (no. of patients)	Percentage of patients dead at 3 months, according to cause of death		
	Asper-gillosis	Underlying disease	Other/ unknown
Disease classification			
Aspergillus colonization (508)	<1	3	9
IA (148)	40	10	12
Risk			
Allogeneic BMT (39)	39	10	8
Autologous BMT (14)	29	7	0
Neutropenia (61)	34	16	13
Hematologic cancer (106)	27	12	11
Solid-organ cancer (124)	5	3	18
Corticosteroid use (381)	11	7	15
Treatment for IA			
Amphotericin B (95)	38	11	11
Itraconazole (43)	21	7	2

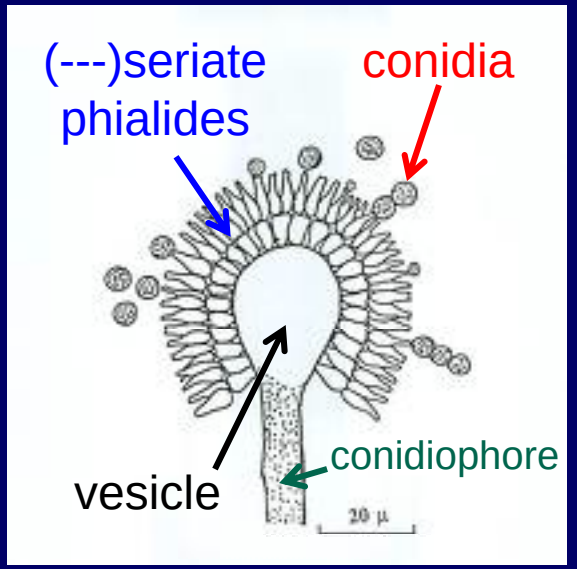
NOTE. BMT, bone marrow transplant; IA, invasive aspergillosis.

Clin. Infect. Dis. **33:** 1824-1833; 2001

ASPERGILLOSIS

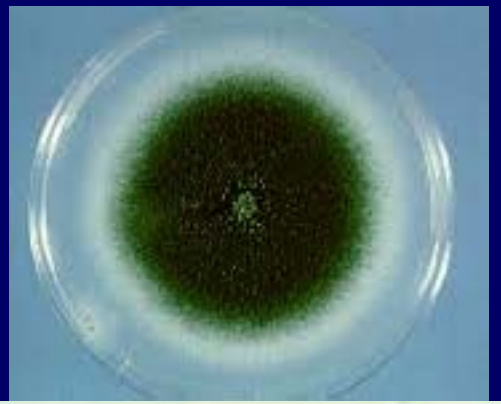






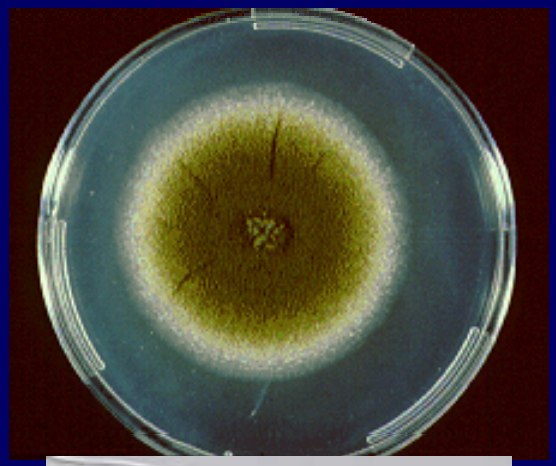
Aspergillus fumigatus

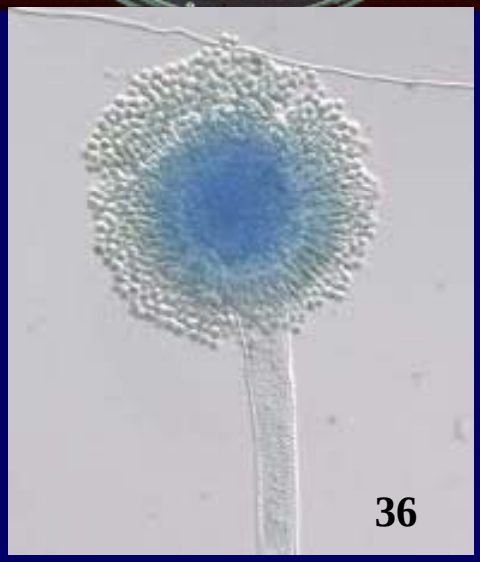
- Maturity in ~3 days
- Conidiophores short & smooth
- Colony surface becomes dark greenish to gray; reverse white to tan
- Uniseriate phialides on upper 2/3 of vesicle; parallel to axis of conidiophore



Aspergillus flavus

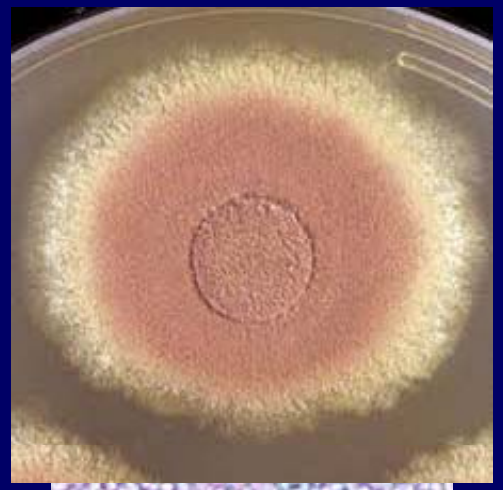
- Commonly associated with aflatoxins
- Conidiophores rough & spiny
- Colony surface velvety, yellow to green or brown; reverse white to tan
- Uniseriate and biseriate phialides covering entire vesicle (all directions)

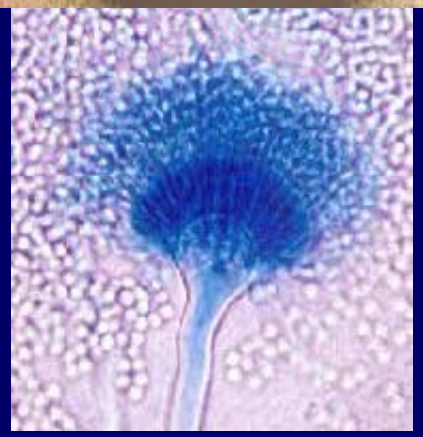




Aspergillus terreus

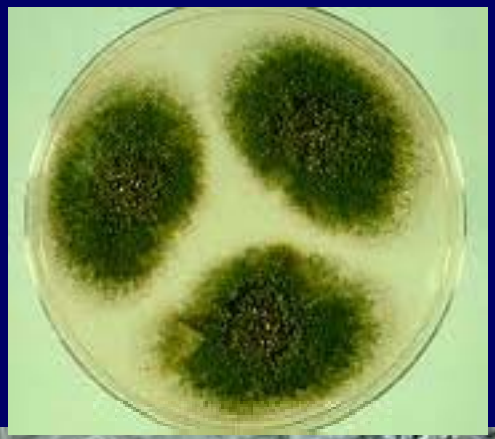
- Commonly considered contaminant
- Conidiophores short & smooth
- Colony surface velvety, cinnamon brown; reverse white to brown
- Biseriate phialides very compact; can be quite lengthy





Emericella (*Aspergillus*) *nidulans*

- Commonly considered contaminant
- Conidiophores short, smooth, brown
- Colony surface typically green (yellow in spots); reverse purplish red
- Biseriate, short, columnar phialides; cleistothecia, Hülle cells

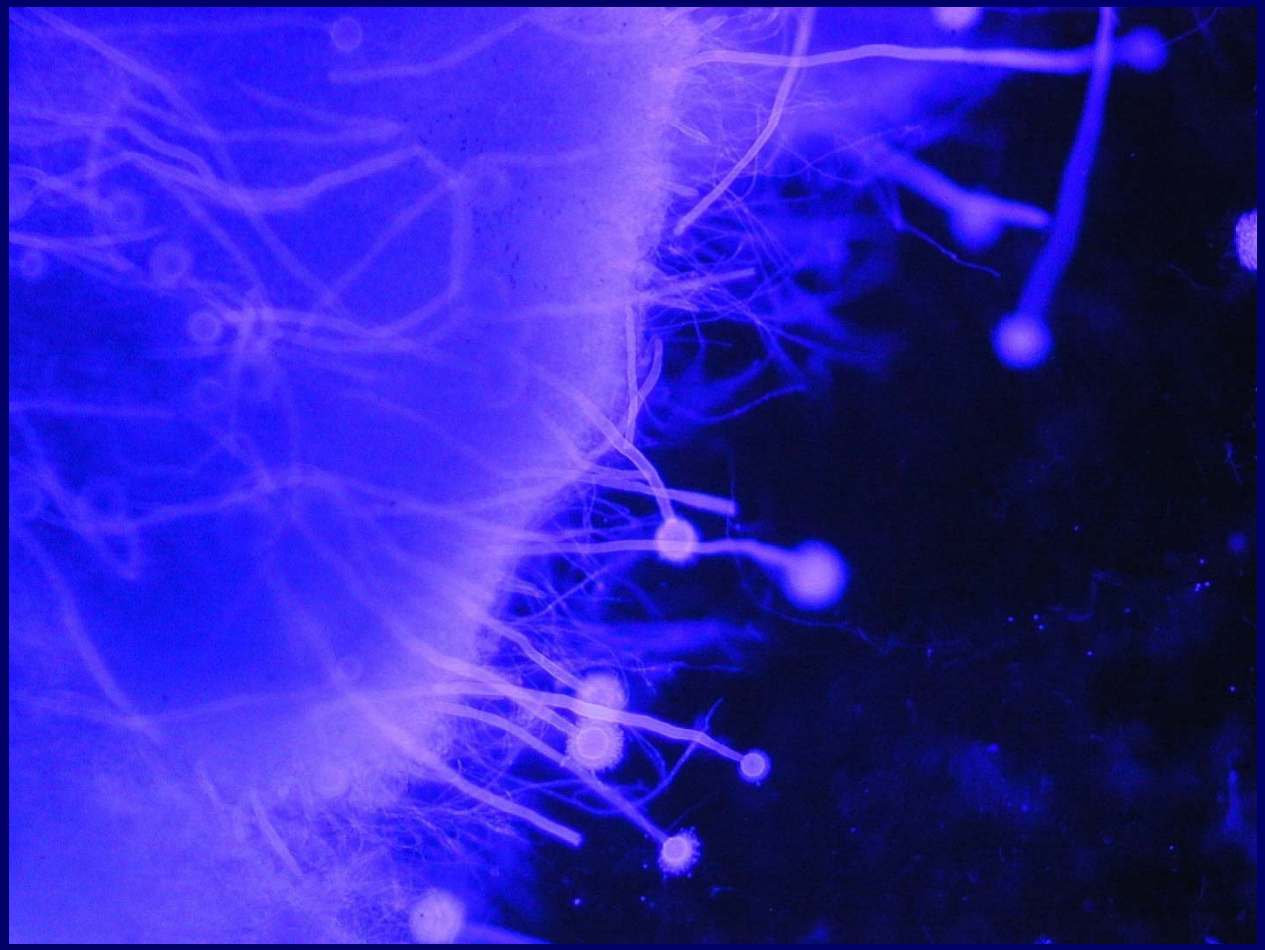


CASE PRESENTATION

- 19-year-old male with three-day history of congestion and ear pain
- PMH of psoriasis in multiple cutaneous sites; daily ibuprofen for tonsillar hypertrophy
- Previous regimens of amoxicillin-clavulanate, amoxicillin, otic neomycin-polymyxin, otic ciprofloxacin-hydrocortisone
- Pain worsened; ENT consult

Courtesy T. K. Block

CASE PRESENTATION

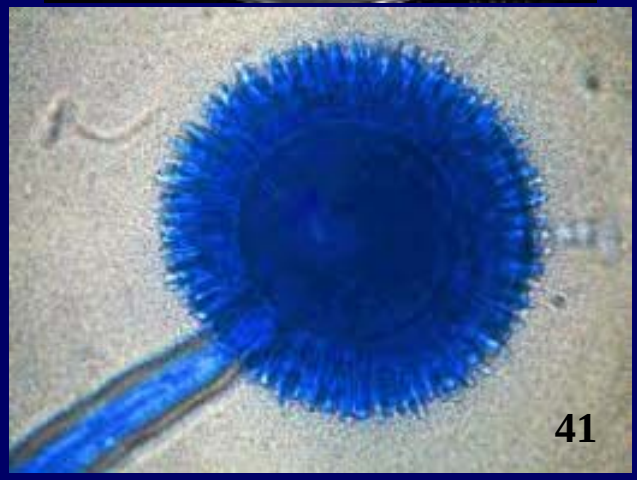


calcofluor white stain;
400x total magnification

Courtesy T. K. Block

Aspergillus niger

- Can cause disease in debilitated patients
- Conidiophores long & smooth
- Colony surface starts white to yellow, turns black; reverse white to yellow
- Biseriate phialides; forms a “radiate head”



Aspergillus niger OTOMYCOSIS

- *A. niger* at least two times more common than *A. flavus* in context of otomycosis

Eur. J. Clin. Microbiol. Infect. Dis. **8:** 413-437; 1989
Am. J. Trop. Med. Hyg. **29:** 620-623; 1980

- Superficial infection; immunocompetent hosts

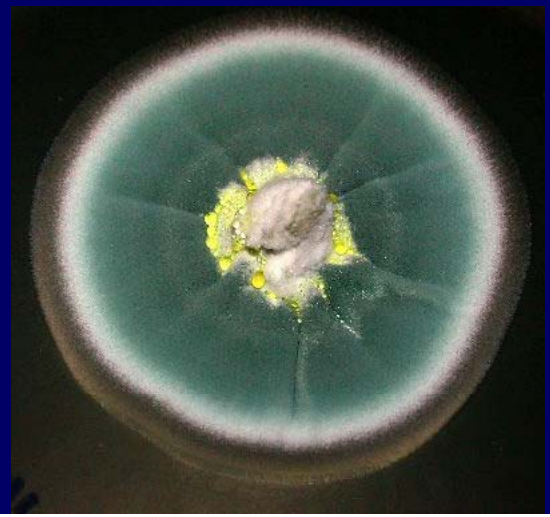
Eur. J. Clin. Microbiol. Infect. Dis. **8:** 413-437; 1989

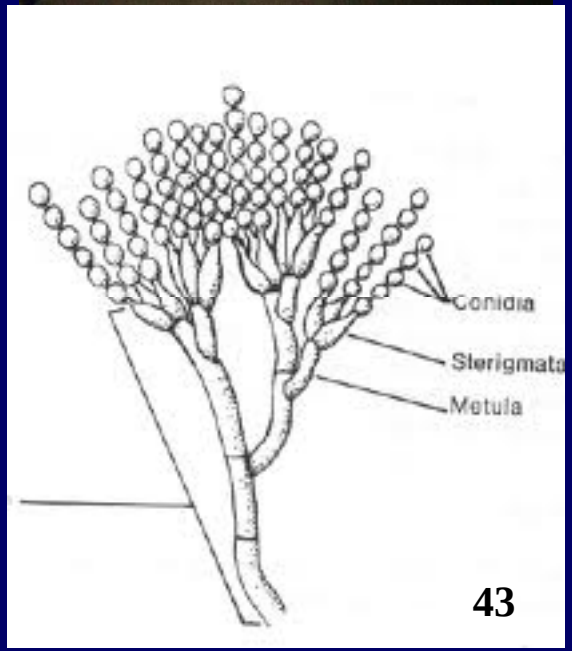
- Self-manipulation; manipulation by barbers

Am. J. Trop. Med. Hyg. **29:** 620-623; 1980

Penicillium spp.

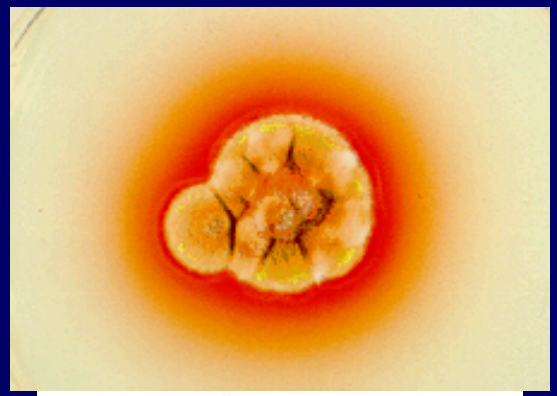
- Typically contaminant; ear, respiratory, cornea, endocarditis
- Maturity in ~4 days
- Colony surface becomes powdery and bluish green with white border; reverse variable
- Branched or non-branched conidiophores; secondary branches known as metulae

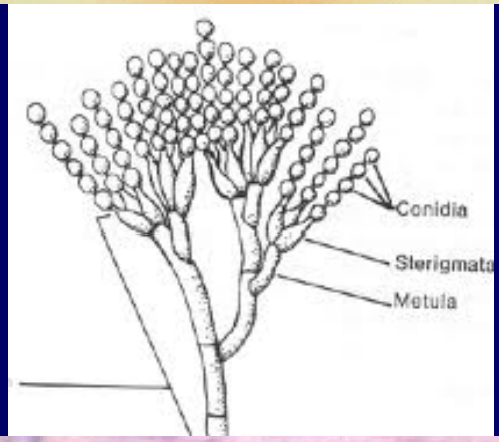




Penicillium marneffei

- Endemic to Southeast Asia; compromised and competent
- Mold maturity (25° C) in ~3 days
- Colony surface can become reddish yellow with light edge; reddish pigment diffusion
- Yeastlike cells observed at 35-37° C; central cross wall as result of fission (not budding)



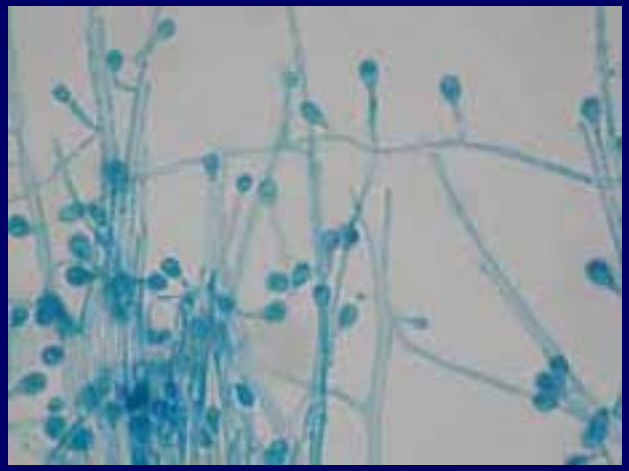


Fusarium spp.

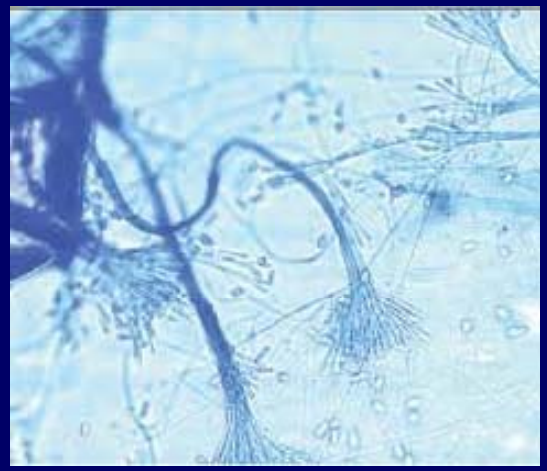
- Common contaminant; mycotic keratitis, disseminated disease
- Maturity in ~4 days
- Cottony surface, develops violet or pink center with light periphery; reverse light
- Canoe-shaped macroconidia ± oval 1- to 2-celled conidia in clusters resembling *Acremonium*

Pseudallescheria HOLOMORPH

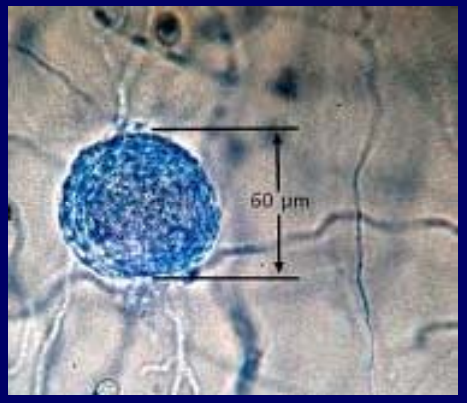
- Mycetoma; respiratory/sinus, disseminates (bone, brain, eyes, meninges)
- Maturity in ~7 days; mouse-like appearance



Scedosporium apiospermum
asexual
no inhibition by cycloheximide



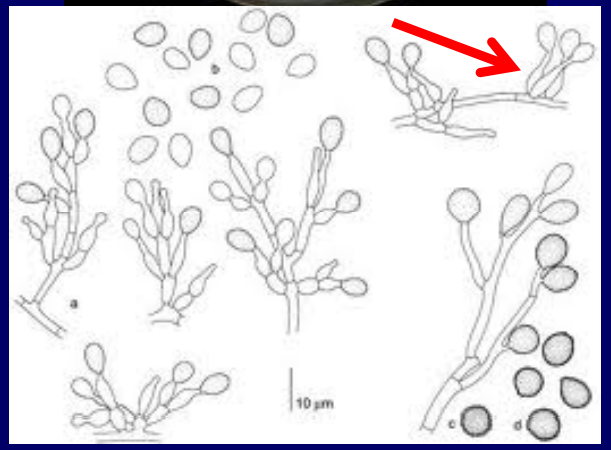
Graphium
asexual
no inhibition by cycloheximide



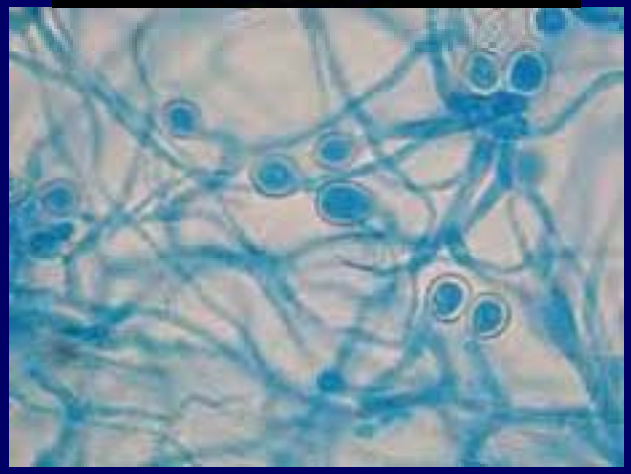
Pseudallescheria boydii
sexual
inhibited by cycloheximide

Scedosporium prolificans

- Invasive infection (osteomyelitis, arthritis); competent & compromised
- Maturity in ~5 days; growth inhibited by cycloheximide
- Cottony or moist surface, becomes dark gray/black with white tufts; reverse gray/black
- Olive to brown conidia, ovoid; annellides have swollen base and elongated neck



MORE MIMICRY



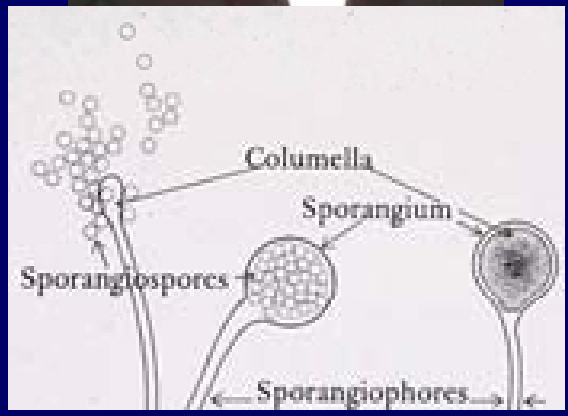
Chrysosporium spp.

Sepedonium spp.

Malbranchea spp.

MUCORMYCOSIS

- Rapid growers
- Diabetic susceptibility
- Different cellular nomenclature
- Ribbon-like hyphae;
  most are aseptate



- www.youtube.com/watch?v=IK0MtXNKgKI

MUCORMYCOSIS

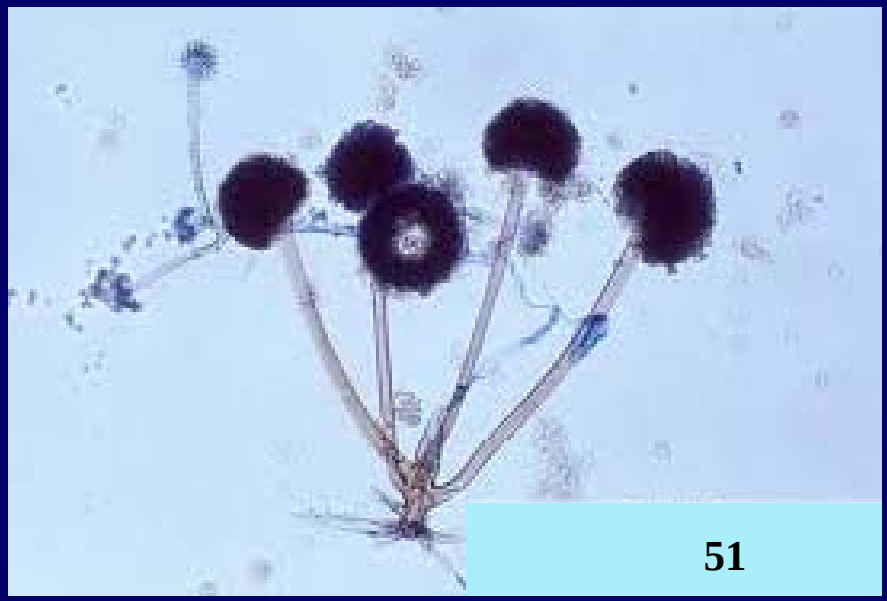
Organism isolated	No. (%) of all patients	No. of patients who died/total no. with the organism (%)
Rhizopus species	218 (47)	105/218 (48)
Not speciated	125 (27)	61/125 (49)
R. oryzae	55 (12)	26/55 (47)
R. rhizopodiformis	20 (4)	9/20 (45)
R. microsporus	11 (2)	7/11 (64)
R. nigricans	7 (2)	1/7 (17)
R. stolonifer	1 (1)	1/1 (100)
Mucor species	85 (18)	44/85 (52)
Cunninghamella bertholletiae	34 (7)	26/34 (76)
Apophysomyces elegans	27 (6)	6/27 (22)
Absidia species	25 (5)	8/25 (32)
Saksenaea species	21 (5)	9/21 (43)
Rhizomucor pusillus	19 (4)	10/19 (53)
Entomophthora species	13 (3)	2/13 (15)
Conidiobolus species	10 (2.2)	5/10 (50)
Basidiobolus species	9 (2)	3/9 (33)
Cokeromyces species	3 (0.6)	1/3 (33)
Syncephalastrum species	1 (0.2)	0/1 (0)

Type of infection, by site	Proportion (%) of all patients	No. of patients with the infection who died/total no. with the infection (%)
Sinus		
Overall	359/929 (39)	165/359 (46)
Rhinocerebral[a]	196/929 (21)	122/196 (62)
Sino-orbital	74/929 (8)	18/74 (24)
Sinusitis	74/929 (8)	12/74 (16)
Sinopulmonary	15/929 (2)	13/15 (87)
Pulmonary		
Overall	224/929 (24)	170/224 (76)
Localized	121/224 (54)	73/121 (60)
Deep extension	15/224 (7)	13/15 (87)
Disseminated	88/224 (39)	84/88 (95)
Cutaneous		
Overall	176/929 (19)	54/176 (31)
Localized	98/176 (56)	10/98 (10)
Deep extension	43/176 (24)	11/43 (26)
Disseminated	35/176 (20)	33/35 (94)
Cerebral		
Overall	87/929 (9)	69/87 (79)
Localized[a]	45/87 (52)	28/45 (62)
CNS dissemination	42/87 (48)	41/42 (98)
Gastrointestinal	66/929 (7)	56/66 (85)
Generalized disseminated	25/929 (3)	25/25 (100)
Kidney	22/929 (2)	9/22 (41)
Other solid organ	15/929 (2)	9/15 (60)

Clin. Infect. Dis. **41:** 634-653; 2005

Rhizopus spp.

- Common contaminants
- Maturity in ~4 days; growth inhibited by cycloheximide
- Cotton candy--white at first, then gray or yellowish brown;
  reverse white
- Rhizoids opposite of sporangiophores



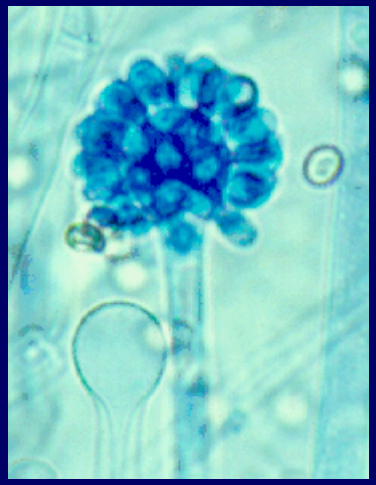
Mucor spp.

- Common contaminant
- Maturity in ~4 days; growth inhibited by cycloheximide
- Cottony or moist surface, becomes gray; reverse white
- Rhizoids absent

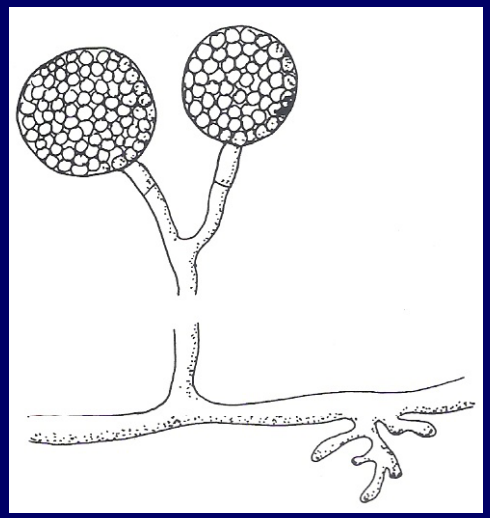
Lichtheimia spp.

- Common contaminant
- Maturity in ~4 days; growth inhibited by cycloheximide
- Coarse, wooly-gray surface--eventually covers surface with “fluff”; reverse white
- Sporangiophores form conical apophysis just below columella; rhizoids alternate

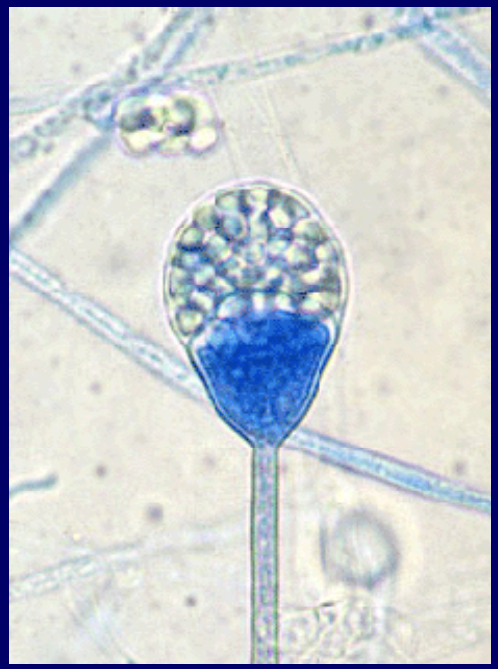
OTHER MUCORMYCETES



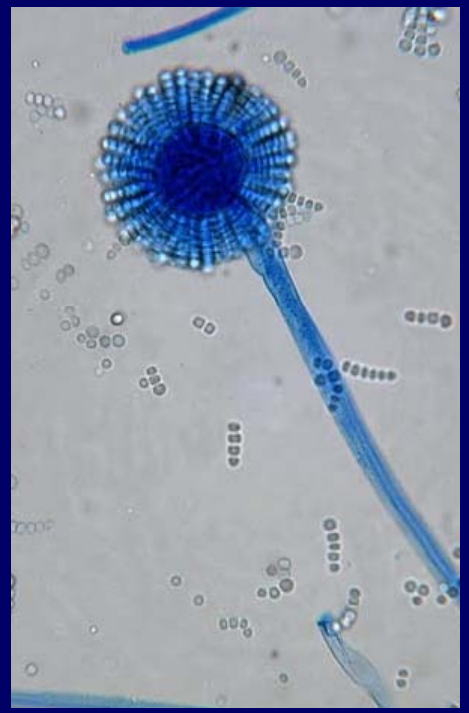
Cunninghamella spp.



Rhizomucor spp.



Apophysomyces spp.



Syncephalastrum spp.

Antifungal Susceptibility Testing

CLSI DOCUMENTS OF INTEREST

- M38-A2 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi, 2nd ed.; Approved Standard
- M51-A Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi; Approved Guideline

BROTH MICRODILUTION

- "Intended for testing common filamentous... moulds, including the dermatophytes, which cause invasive and cutaneous infections, respectively..."

Aspergillus spp.
Rhizopus spp.
Scedosporium prolificans
Fusarium spp.
Pseudallescheria boydii
Sporothrix schenckii (mould)
Opportunistic monilaceous fungi
Opportunistic dematiaceous fungi

- "Method has not been used in studies of the yeast or mould form of dimorphic fungi."

BROTH MICRODILUTION

- RPMI 1640 broth (MOPS buffer, 0.2% dextrose)
- 7-day filamentous fungus growth;
  potato dextrose agar slants
- Flood with saline
  Withdraw mixture, particles settle 3-5 min
  Upper suspension contains mycotic elements
- Inoculum (OD_{530}) dependent upon fungus [0.09-0.30]; range of 0.6 to 3.0 x 10^6 CFU/mL

BROTH MICRODILUTION

Non-dermatophyte filamentous fungi

0.03-16 μg/mL	amphotericin B	ravuconazole
	ketoconazole	itraconazole
	posaconazole	voriconazole
0.125-64 μg/mL	flucytosine	fluconazole
0.015-8 μg/mL	flucytosine	fluconazole

CLSI M38-A2

BROTH MICRODILUTION

Dermatophytes

0.06-32 μg/mL	ciclopirox
0.125-64 μg/mL	griseofulvin fluconazole
0.001-0.5 μg/mL	itraconazole voriconazole terbinafine
0.004-8 μg/mL	posaconazole

CLSI M38-A2

BROTH MICRODILUTION

- 35° C ambient air

  21-26 hours for mucormycetes
  70-74 hours for *Scedosporium* spp.
  46-50 hours for most others

  21-26 hours for echinocandin testing
  46-72 hours for *Scedosporium* spp./echinocandins

- Amphotericin B: observe 100% inhibition
  Other agents: observe 50% inhibition
  Dermatophytes: observe 80% inhibition

  Echinocandins: lowest concentration resulting in small, compact, rounded hyphae
  Minimum Effective Concentration (MEC)

CLSI M38-A2

Etest

- Not FDA-approved for filamentous fungi
- Etest MIC and broth microdilution data more comparable for trizoles (>90% agreement) than for amphotericin B (>80% agreement)
- Etest MIC values higher for *S. apiospermum*, *A. flavus*, *S. prolificans* higher than reference values

J. Clin. Microbiol. **39:** 1360-1367; 2001

CLINICAL UTILITY

- "Factors related to…..appear to have more value than the MIC as predictors of clinical outcome."

Clin. Infect. Dis. **24:** 235-247; 1997

- "Very few correlations of in vitro results with in vivo response have been reported for mold infections."

Curr. Fungal Infect. Rep. **3:** 133-141; 2009

- "…tests are currently most useful for detecting resistance or outliers based on either assigned in vitro breakpoints or epidemiological cutoffs."

Pfaller *et al*., Manual of Clinical Microbiology, tenth ed.

THE END

- Mostly an observational science (occasional biochemical may help with dermatophytes); note growth distribution and rate of growth
- Antifungal susceptibility testing for moulds continues to be a work in progress
- See you at the Dells




CREDITS

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