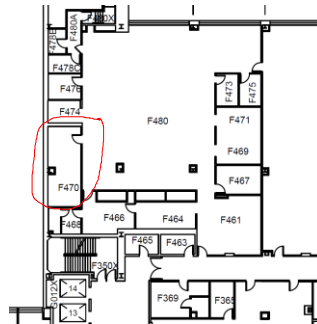
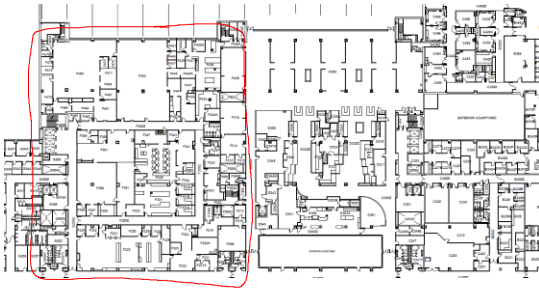
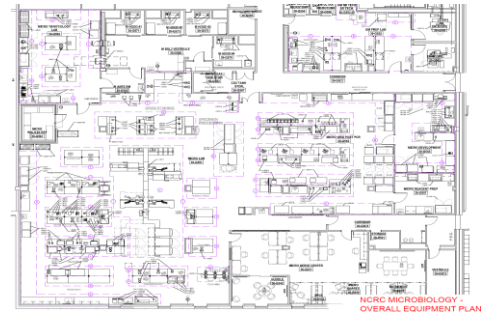
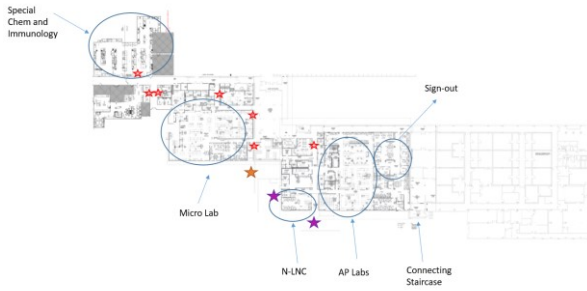
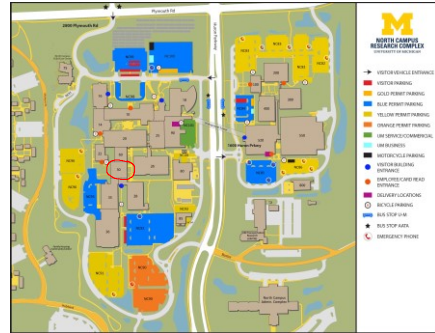
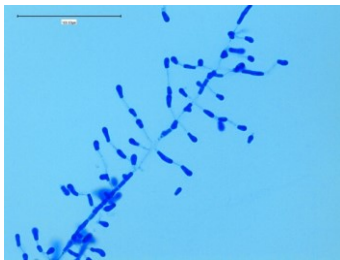
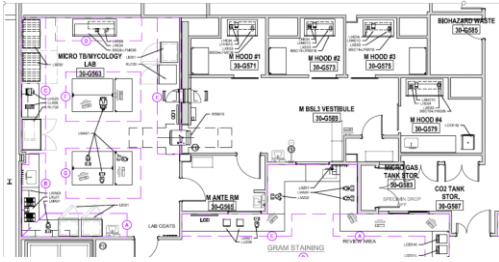


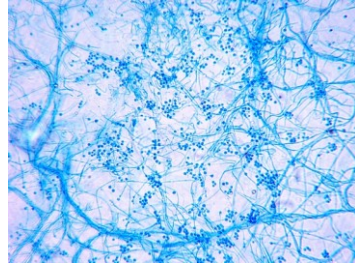
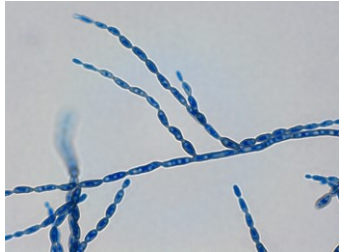
Fungal Imposters

Peggy Mahlmeister MLT (ASCP)
Clinical Microbiology Supervisor
Michigan Medicine
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Mycology

Plated media – initial culture processing

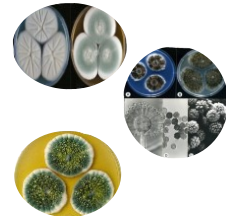
BHI Blood
 IMG
 BCG
 SAB
 Direct smear (calcofluor white)

Incubate at 28 -30 C
 4 weeks
 Read plates daily first week
 Read plates weekly weeks 2-4



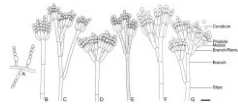
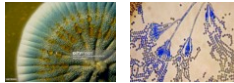
Mycology

- Positive culture work up
 - Molds
 - Surface color
 - Reverse color
 - Microscopic examination
 - Lactophenol Acridine Blue
 - Scotch tape prep
 - Slide culture



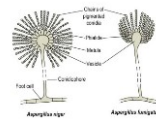
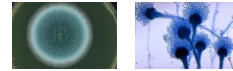
Penicillium

- Commonly considered contaminants
- Have been found to cause corneal, cutaneous, external ear, respiratory and urinary tract infections and endocarditis after insertion of valve prosthetics
- Rapid growth, mature at 4 days
- Bluish green with a white border
- Septate hyphae, branched or unbranched conidiophores that have secondary branches called metulae
- Flask shaped phialides that bear unbranched chains of smooth or rough conidia
- The entire structure forms the characteristic "penicillus" or "brush" appearance



Aspergillus

- 180 species of aspergillus are known; six, less than 1% of these are known to cause disease.
- Commonly found as a contaminant due to the Aspergillus species being widespread in the environment. Causes Aspergillosis in the form of:
 - Rapid growth, mature within 3 days
 - Surface starts out white and the very inside of green, yellow, orange, brown, or black depending on the species, texture is sandy. Reverse is usually white, golden or brown.
 - Septate hyphae, unbranched conidiophores arise from a foot cell, conidiophore is a single cell that forms a vesicle vesicle. Vesicles are large and completely covered with flask shaped phialides, which may develop directly on the vesicle (uniseriate) or on cells known as the metulae (biseriate). The phialides produce chains of conidia.



Aspergillus fumigatus

- The most common cause of invasive disseminated aspergillosis, allergic aspergillosis and fungal sinusitis
- Surface is velvety or powdery, vesicles shades of green with a white border turning dark with age. Reverse white to tan.
- Smooth conidiophores, relatively short
- Phialides are uniseriate, formed closely together (compact), forming only on the upper 2/3 of the vesicle, parallel to the axis of the conidiophore (eomerian)
- Conidia are smooth or slightly rough and round



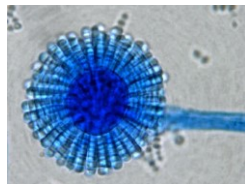
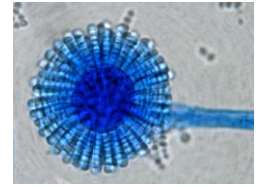
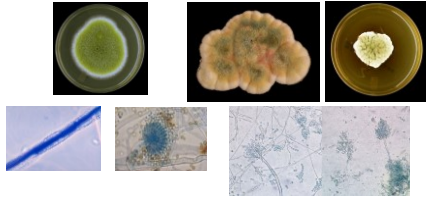
Aspergillus Niger

- Most commonly isolated from the external ear, causing otomycosis
- Frequent agent of fungal balls in preexisting pulmonary cavities.
- Sometimes causes disseminated disease
- Rapid growth
- Surface is black with a white border, sometimes starting out as yellow when young
- Reverse is white to cream
- Conidiophores are long, smooth and may be brownish towards the top
- Phialides radiate around entire vesicle and are biserial with the metulae hence at long as the phialides.
- Conidia are rough and dark



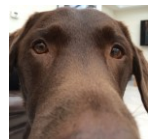
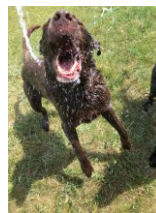
Aspergillus flavus

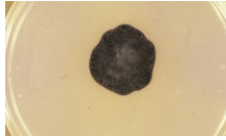
Aspergillus versicolor



Syncephalostrum spp
Rapid growth
Zygomycete
Sporangiophores terminate in vesicle covered with finger like tubular sporangia containing chains of round spores.

Dematiaceous





Lateral and Transverse Septa
Chaining Conidia



Alternaria spp

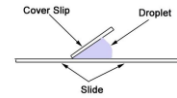
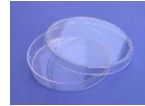




Germ Tube Test for Differentiation of some Dematiaceae Fungi

Used to help differentiate between Bipolaris, Drechslera and Exserohilum

- Place a drop of water on a microscope slide
- Inoculate the drop with a small amount of actively growing fungus (Examine the slide to make sure that conidia are present)
- Place a coverslip over suspension
- Incubate in a moist chamber at room temperature for 8-24 hours
- Examine slide to determine origin and orientation of germ tubes.



Key Features for Differentiation

STRUCTURE	DIFFERENTIATION
Fusoid to cylindrical conidia that are evenly pigmented	Bipolaris (+) Carrisoria* (+)
Distoseptate conidia	Bipolaris (+) Carrisoria** (+)
Profuse conidiation	Bipolaris (+) Drechslera (-)
Slight protrusion at conidial hilum	Bipolaris (+) Drechslera (-)
Germ tube perpendicular to conidial axis	Bipolaris (-) Drechslera (-)
Germ tube originating from any cell of conidium (not only from terminal cell)	Bipolaris (-) Drechslera (-)
>5 septa in conidia	Bipolaris (-) Exserohilum (+)
Strong protrusion at conidial hilum	Bipolaris (-) Exserohilum (+)
Genuclate sympodial conidiophores	Bipolaris (+) Helminthosporium*** (-)
Conidia which are larger at the base (obclavate in shape)	Bipolaris (-) Helminthosporium (+)



Bipolaris spp: Profuse conidiation; 3-5 septa, slightly protruding hilum

Drechslera spp: Poor conidiation; 3-5 septa, hilum does not protrude



Bipolaris spp: 3-5 Septa Germ Tube: 1 or both ends along axis of conidium



Exserohilum spp: 5-12 Septa Germ Tube: 1 or both ends along axis of conidium



Drechslera spp: 3-5 Septa Germ Tube perpendicular to conidial axis



Helminthosporium spp: Conidia are large, dark, thick walled and contain 6 or more cells

Curvularia spp: Conidia are large, usually containing 4 cells that will eventually appear curved due to swelling of a central cell.

Exserohilum spp: Conidia are brown, thick walled with 7-11 septa. The hilum protrudes from the conidium



On 18 September 2012, a clinician at Vanderbilt University Medical Center notified the Tennessee Department of Health of a 56-year-old male patient with culture-confirmed *Aspergillus fumigatus* meningitis. It had been diagnosed 46 days after an epidural steroid injection at a Tennessee ambulatory surgical center that resulted in his death on hospital day 22. Within 9 days, a collaborative initial investigation identified an additional eight patients with clinically diagnosed, culture-negative meningitis (seven in Tennessee and one in North Carolina).

Intense epidemiologic investigation also revealed that all nine patients had received one or more injections from three specific lots of preservative-free methylprednisolone acetate solution (MPA) prepared at the New England Compounding Center (NECC) in Framingham, MA. The first of the three lots was produced by NECC on 21 May 2012 and was voluntarily recalled by NECC on 26 September. Before the recall, approximately 17,500 vials from the contaminated lots had been distributed to 75 facilities in 23 states. The MPA had been used to treat peripheral joint and back pain. On 3 October, the recall was expanded to all lots of MPA, along with all lots of any other sterile products intended for intrathecal injection; this was followed, on 6 October, by a further recall of all remaining NECC products.

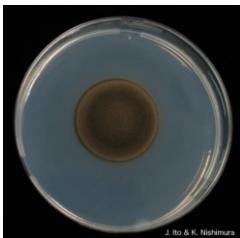
Approximately 14,000 patients had potentially been exposed to MPA from at least one of the three implicated lots and were considered at risk for infection. Of the exposed patients, 89% received spinal injections and 12% received joint injections. As of 5 August 2013, 749 cases of infection had been reported in 20 states, with the largest numbers occurring in Michigan, followed by Tennessee, Indiana, Virginia, and New Jersey.

State	Total cases	Michigan only	Michigan + Pennsylvania	Michigan + Ohio + West Virginia	Pennsylvania only	Pennsylvania + Ohio + West Virginia	No. of deaths
Florida	25	23	2	2	1	0	7
Georgia	2	2	0	0	0	0	0
Idaho	1	1	0	0	0	0	0
Illinois	2	2	0	0	0	0	0
Indiana	44	38	17	0	24	0	13
Iowa	28	28	0	0	0	0	13
Michigan	388	388	0	0	0	0	117
Minnesota	127	127	0	0	0	0	17
North Carolina	162	1	3	0	168	0	1
New Hampshire	14	0	0	0	0	14	0
New Jersey	10	10	0	0	0	0	0
New York	1	0	0	0	1	0	0
Ohio	28	12	0	0	16	0	7
Pennsylvania	1	0	0	0	0	1	0
Rhode Island	1	0	0	0	0	1	0
Tennessee	15	0	0	0	0	15	0
Texas	1	0	0	0	0	1	0
Virginia	14	14	0	0	0	0	0
West Virginia	7	0	0	0	0	7	0
Total	548	238	100	7	100	30	141

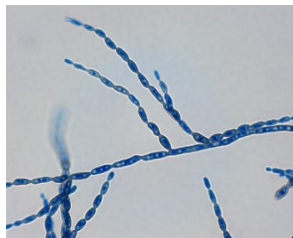
The CDC developed a novel test using PCR and DNA sequencing to rapidly detect *Exserohilum rostratum* and other fungal DNA in a variety of specimens. For *E. rostratum*, the assay demonstrated a specificity of 100% and a sensitivity of 29%. This is an improvement over culture, which, for *E. rostratum*, was found to have a sensitivity of only 14%. Although the first patient reported was infected with *A. fumigatus*, the predominant fungus detected in the majority of cases was *E. rostratum*. As of 8 July 2013, *E. rostratum* had been identified in 153 case patients, while in a relatively few cases ($n < 27$), 12 other genera of fungi were recovered and identified. Multiple genera were detected in several cases. The additional fungi encountered were: *Aspergillus* spp., *Cladosporium* spp., *Alternaria* sp., *Bipolaris* sp., *Chaetomium* sp., *Epicoccum nigrum*, *Malassezia restricta*, *Paeclomyces* sp., *Penicillium* sp., *Scopulariopsis brevicaulis*, *Stachybotrys chartarum*, and *Coelomycetes* fungi

Exserohilum sp. was the identity of the fungus in 100 (90%) of the fungus-positive cases.

The fact that in the current outbreak so many cases of serious illness have been caused by a typically uncommon pathogenic fungus is most likely due to the circumstance of the patients receiving an injection of *E. rostratum* directly into the CNS, along with a corticosteroid that reduces the immune response, setting the stage for a "perfect storm"

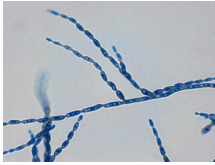


J. Ho & K. Nishimura



K. Nishimura



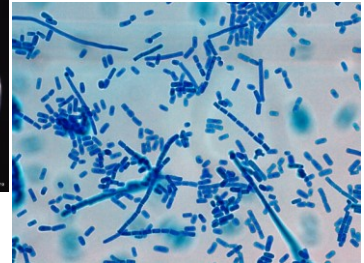
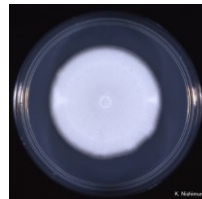
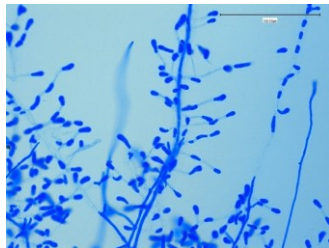


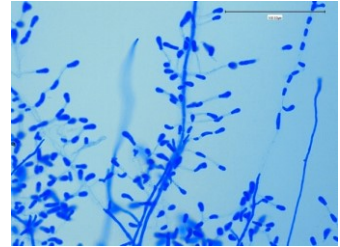
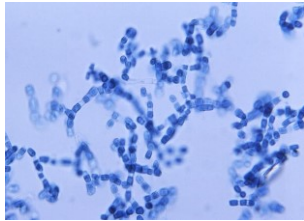
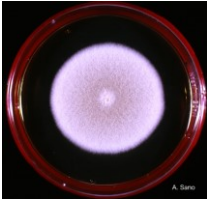
Cladophialophora spp
Slow growth, up to 18 days
Long chains of conidia, pale hila



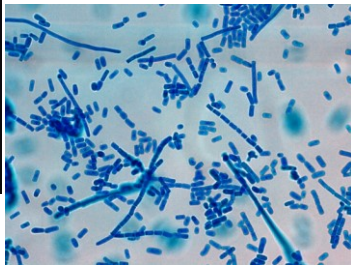
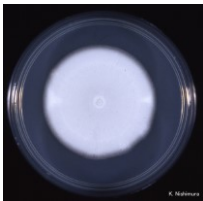
Cladosporium spp
Moderately rapid growth, mature at 7 days
Branching tree like chains, easily dislodged, showing a dark hila
The cells bearing the conidial chains are large, sometimes septate, resembling shields

White Fluffy

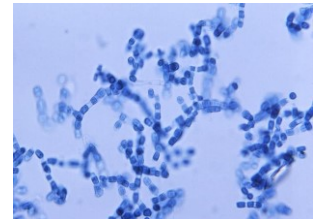
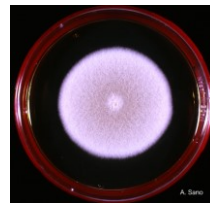




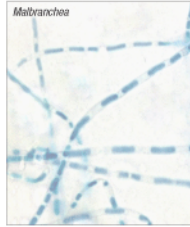
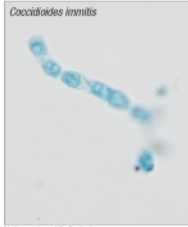
Chrysosporium spp.
 Moderately rapid growth: Mature in 6 days
 Intercalary conidia are sometimes formed; can sometimes be mistaken for alternating barrel shaped conidia. Sometimes a fringe or remnant of supporting cell is left on the base of the conidia when it matures and breaks off of the conidiophore.
 Chrysosporium grows on media with cyclohexamide. If in doubt: DNA Probe



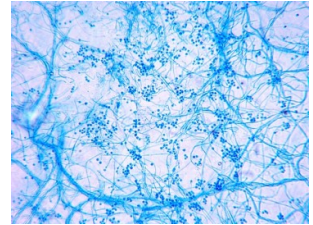
Geotrichum spp
 Rapid growth: Mature at 4 days
 White, yeastlike colonies
 Consecutive formation of arthroconidia



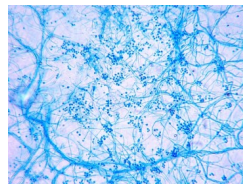
Coccidioides spp
 Moderate rate of growth, mature in 10 days. May take up to 2 weeks for production of arthroconidia.
 Thick walled barrel shaped arthroconidia that alternate with empty cells
 Highly infectious: NO SLIDE CULTURES
 DNA Probe for identification



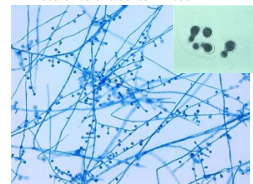
Photos courtesy of Dr. Gordon Lowe

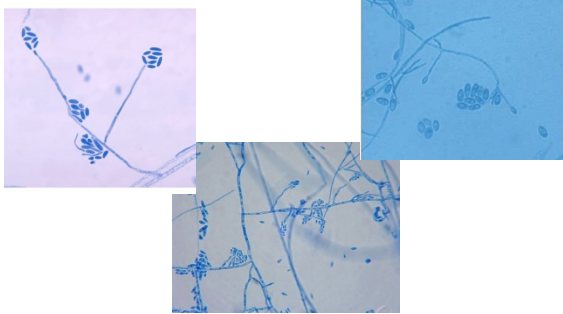


Emmonsia spp
Moderate growth rate, mature in 7-14 days
E. Parva will grow in presence of cyclohexamide

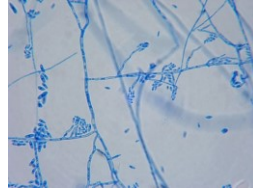


Blastomyces spp
Slow growth rate, sometimes greater than 14 days
Dimorphic: Large broad based budding yeast like cells at 37 on BHI or can be seen on direct smear.
DNA Probe for identification confirmation

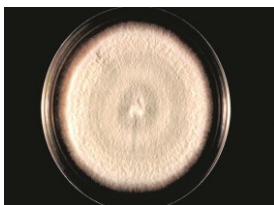




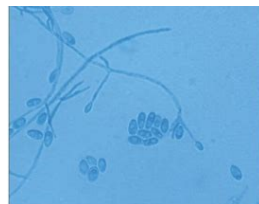
Fusarium spp
 Rapid growth, mature in 4 days. Colonies are white and fluffy that develop a pink or violet center.
 2 types of conidiation: branched and unbranched conidiophores that produce large sickle or canoe shaped conidia long or short simple conidiophores that produce small oval one or two celled conidia singly or in clusters resembling *Acrotonium* spp.

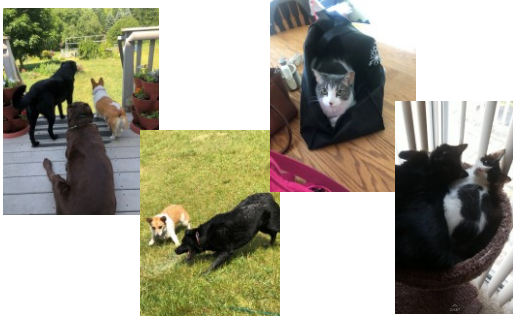
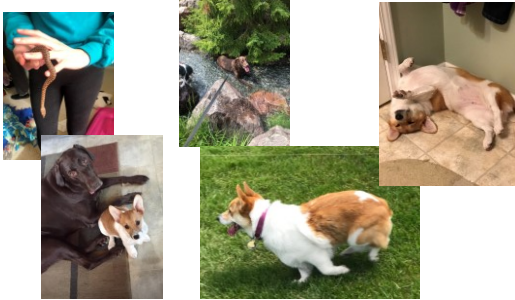


Acrotonium spp
 Moderate growth, mature 5-7 days. Felt like, powdery white, yellow or grayish colony
 Extremely delicate. Phialides are erect, unbranched, tapering and form directly on the hyphae. Most have a septum at the base of the phialide. Usually one celled, sometimes two celled conidia, easily disrupted clusters at tip of phialide.



Phialomonium spp
 Moderately rapid growing, broadly spreading. Surface is white to cream becoming yellowish, grayish with age.
 Phialides form singly along hyphae, no basal septa; may be short or long. Conidia are oval to tear shaped, single celled.






**THANK
YOU**
for
**LISTENING
ANY QUESTIONS?**