MAYO CLINIC

“LET ME SHROOMINATE ON THIS….”

CHALLENGES IN DIAGNOSTIC MYCOLOGY

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DISCLOSURES

- Roche Diagnostics
- Cognex

LEARNING OBJECTIVES

- Overview of diagnostic methods in mycology
- Cases (4)
  - Clinical presentation
  - Laboratory diagnostics
  - Clinical management
  - No *Aspergillus!*

MAYO CLINIC FLORIDA

ESTABLISHED 1986

Multi-disciplinary specialized practice

- 304 beds

Transplants

- Heart, lung, liver, kidney, pancreas
- Approximately 450/yr

Mayo Clinical Laboratories Reference Service

>200 technologists

Microbiology Laboratory

- 47 FTE
- 4 Mycology specialists

OVERVIEW OF DIAGNOSTIC METHODS IN MYCOLOGY

Trichosporon inkin

DIAGNOSTIC METHODS IN MYCOLOGY

14,000 Identified species
700 associated with humans

Culture

Selective and chromogenic media
Recovery variable
Turnaround time
Still gold standard

Morphologic

Subjective and requires skill
Discrepancies with culture

MALDI-TOF

Proteomic
Commercially available
Database dependent

Biochemical

Phenotypic
Commercially available
Automated/manual
Biomarkers

Genomic

Multiplex PCR
Some direct from specimen
Sanger and WGS

Mendonça A, *Res Microbiol*. 2022 Mar-Apr;173(3)

IDENTIFICATION METHODS

CAP F-A 2022

MCF Mycology:

- MALDI-TOF (Bruker) for yeasts
- Morphology for filamentous fungi

System	Freq
API	44
BD Phoenix	16
Mass spectrometry/Bruker MALDI	203
Mass spectrometry/Vitek MS MALDI	172
MicroScan	24
Morphology and Bruker MALDI	89
Morphology and Vitek MS MALDI	80
Morphologic exam/biochemical	27
Remel RapID Yeast Plus	24
Vitek 2	237
Other[a]	26

Yeasts

System	Freq
Biochemical method	12
Mass spectrometry/Vitek MS MALDI	14
Morphology and Bruker MALDI	43
Morphology and sequencing	20
Morphology and Vitek MS MALDI	40
Morphologic exam/biochemical	736
Other[a]	49

Filamentous fungi

CHALLENGES WITH DIAGNOSTIC METHODS

TECHNOLOGY, SPACE, SKILLS, COST

- Number of skilled medical mycologists are dwindling
- Molecular methods (PCR) not standardized
- Limited commercialization
  - LDT vs. RUO vs. IVD
- New technologies
  - Nuclear magnetic resonance – T2 panel
  - Microarrays
  - Solid phase cytometry
  - Spectroscopy

Mendonça A, *Res Microbiol*. 2022 Mar-Apr;173(3)

AND NOW THE CASES…..

CASE #1

WHAT'S IN A NAME (OR NAMES)?

- 54 y.o. male
- CLL and COPD
- Received 1 unit platelets and pRBC previous day
- Presented to ED with severe myalgias, arthralgias
- Cough with whitish sputum
- Admit to ICU, start cefepime, pan-culture evaluation

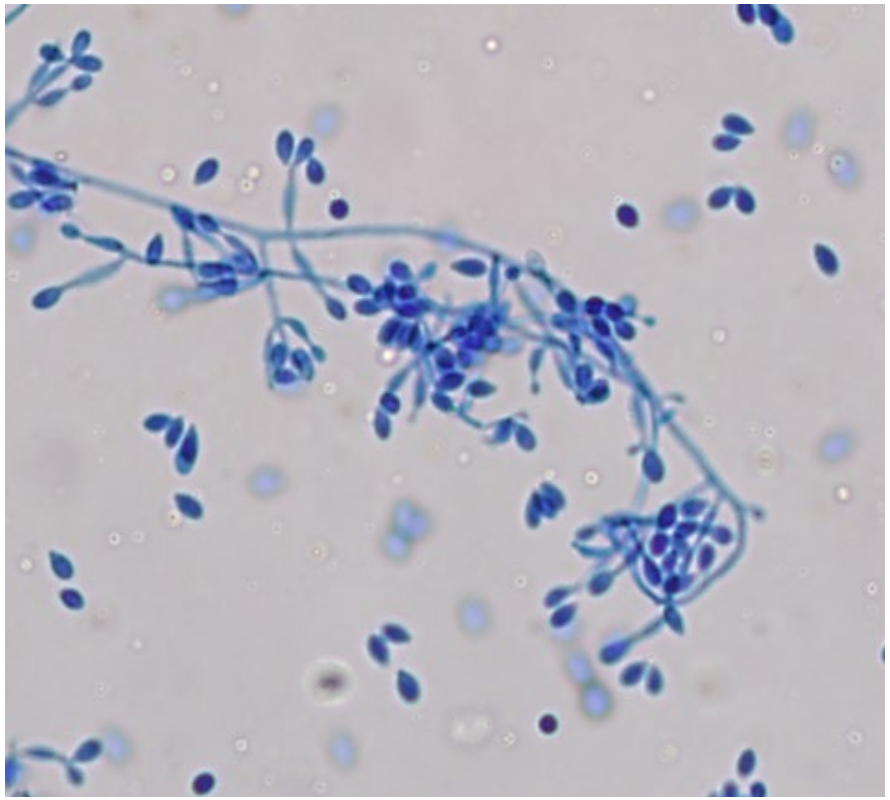
LABORATORY DIAGNOSTICS

- RBC 2.4 x 10^6/mL (4.32 – 5.72 x 10^6/mL)
- Hematocrit 21% (38.8 – 50%)
- WBC 1,700/mL (3,500 – 10,500/mL)
- ANC: 0.4 x 10^9/L
- Blood cultures positive for pan-susceptible *K. pneumoniae*
- Antibiotics adjusted to piperacillin/tazobactam, vancomycin, levofloxacin, valacyclovir, fluconazole

CASE PROGRESSION

- Deterioration in respiratory function over next 72 hours
- CT: bilateral ground glass opacities, micronodules, LLL consolidation
- Antifungal changed to liposomal amphotericin B
- BAL: Many septate hyphae
- 4 days of incubation:
  - Multiple wooly gray colonies
- Patient expired on day 7

MICROSCOPIC MORPHOLOGY



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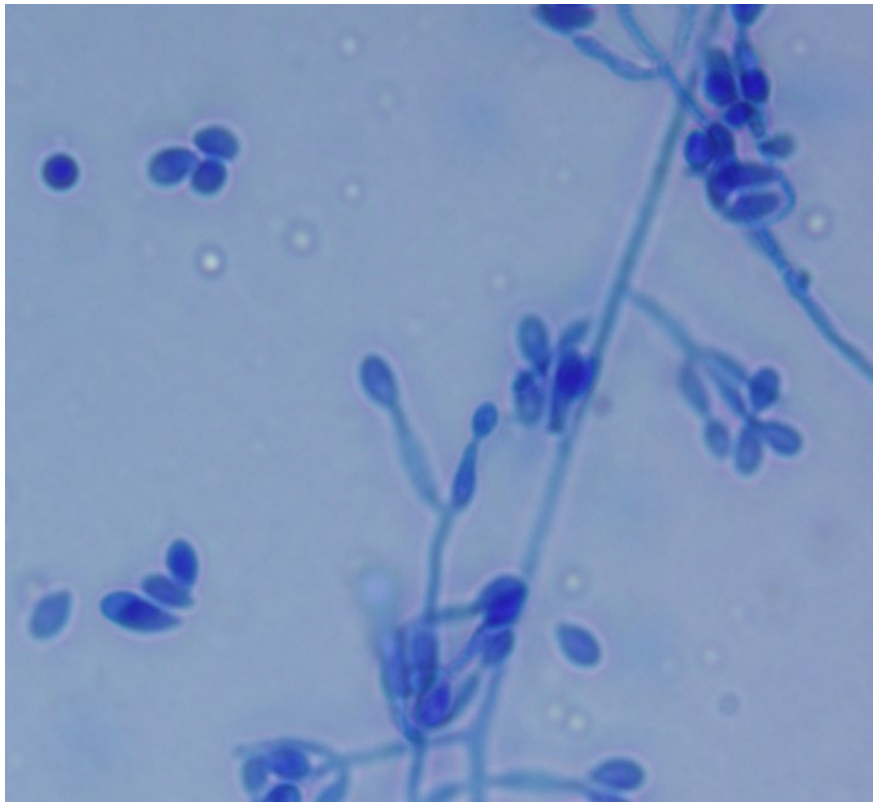


100X

WHAT DO YOU THINK?

(*CUE JEOPARDY MUSIC*)

1

DIAGNOSIS:

PNEUMONIA DUE TO *Lomentospora prolificans*

TAXONOMY: *LOMENTOSPORA PROLIFICANS*

- *Scedosporium inflatum* (1984) + *Lomentospora prolificans* (1974) = *Scedosporium prolificans*
  - Sequencing combined both species as *Lomentospora prolificans* (1990)
- Not to be confused with *Scedosporium apiospermum*!
  - 10 discrete species not including *Lomentospora*

Konsoula A. et al. *Microorganisms* 2022, 10, 1317.

LOMENTOSPORA PROLIFICANS

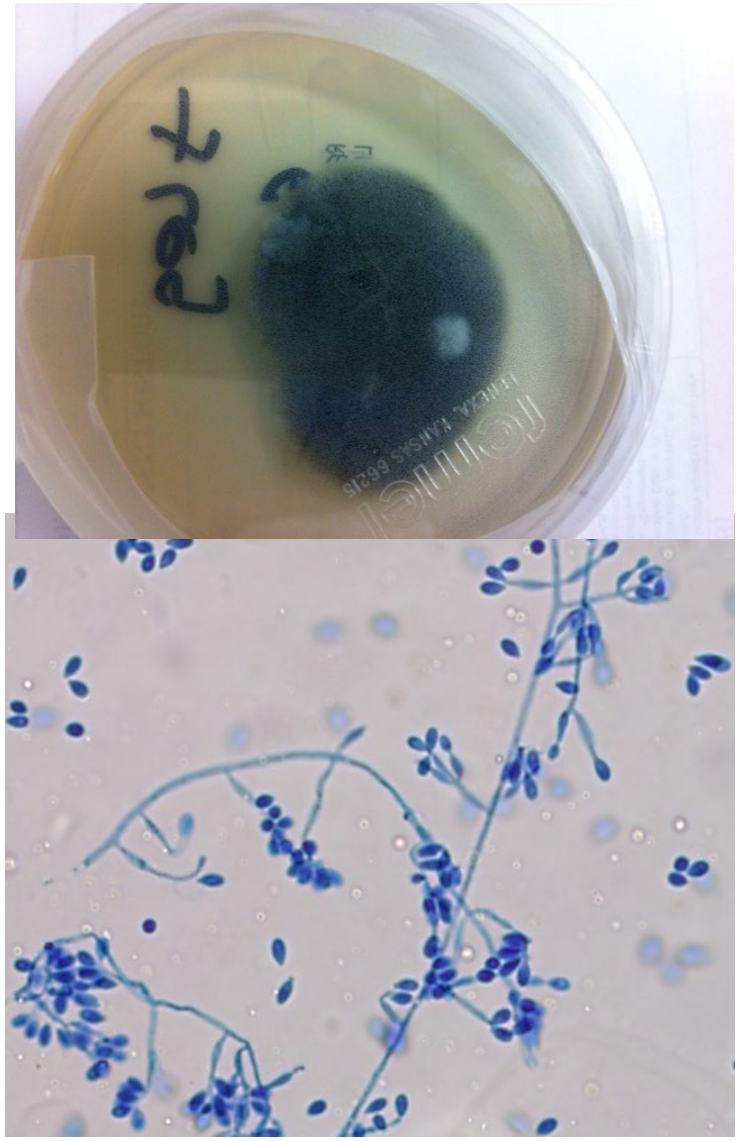
EPIDEMIOLOGY

- Soil saprophyte
- True prevalence not known (not reportable)
  - 2% - 35% of non-*Aspergillus* infections in transplant patients
- Associated with immunocompromised hosts
  - HSCT, SOT, colonization of CF patients
- Disseminated infections, respiratory, endocarditis, CNS/ocular disease
  - 3-month mortality rate 67%

Hoenigl M. *Lancet Infect Dis*. 2021 Aug;21(8):e246-e257.
Bronnimann D. et al. *Medical Mycology*; 59.2021:486–497

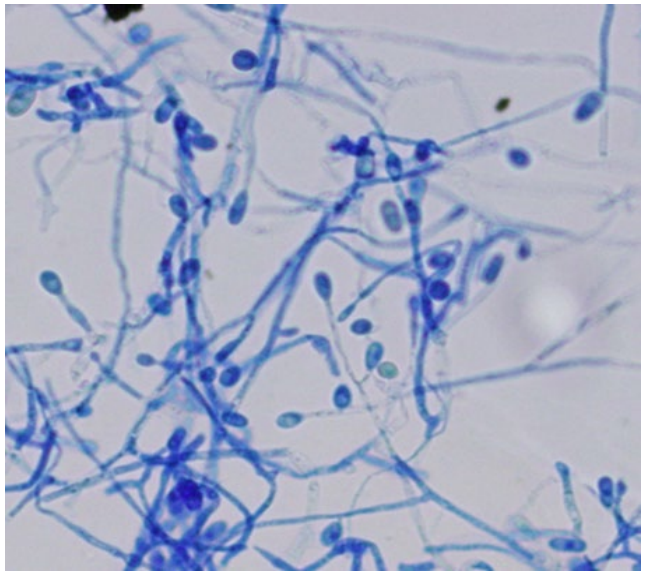
L. PROLIFICANS – CHARACTERISTICS

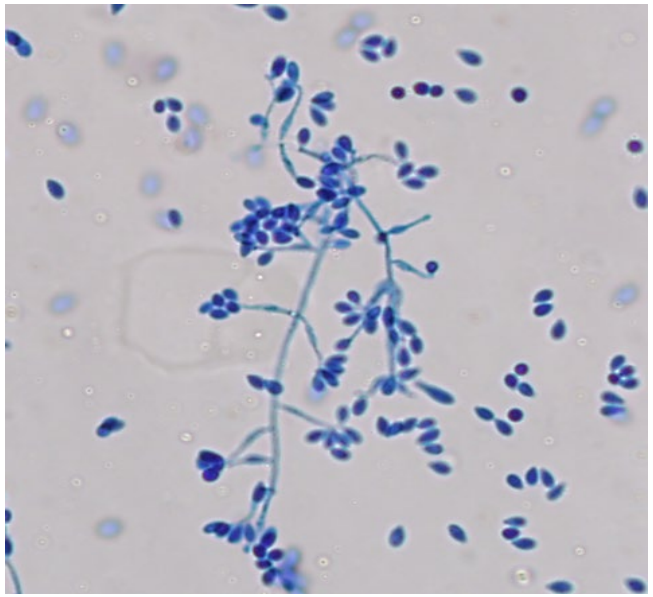
- Growth 3-5 days
- Colonies brown/olive grey/black, with black reverse
- Septate hyphae with 45^{o} branching
- Flask-shaped conidiophores with swollen bases
- Will grow in blood culture bottles
- Cannot grow in presence of cycloheximide

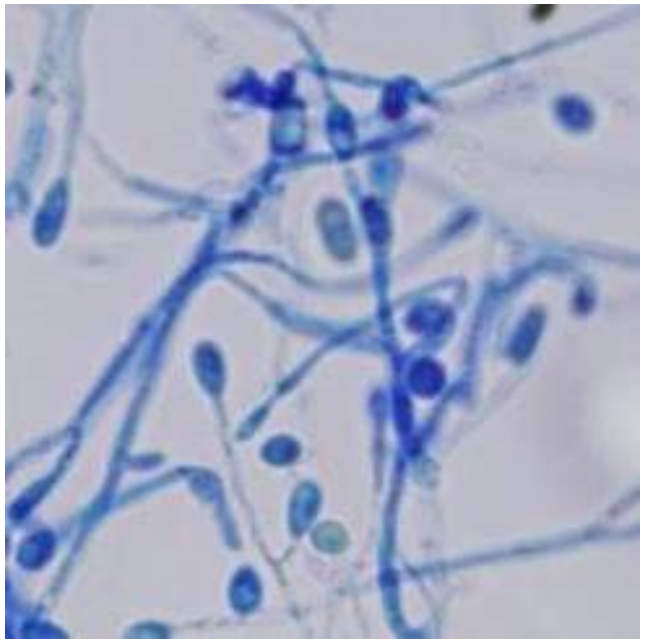


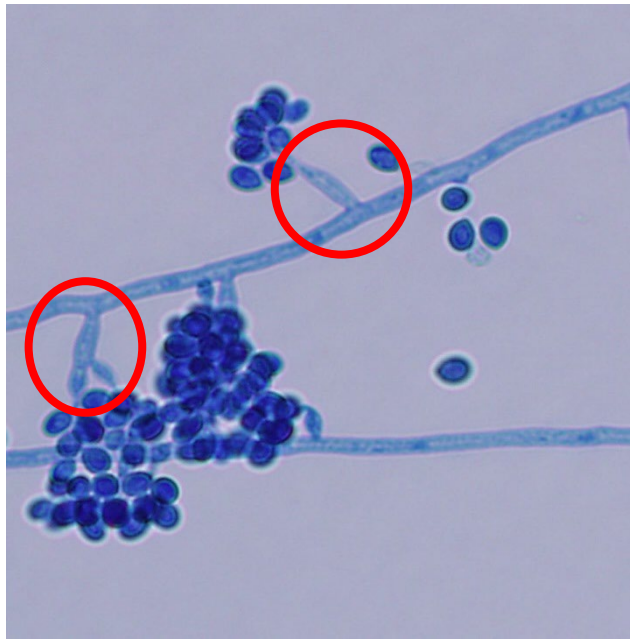
S. apiospermum

- Simple conidiophores with parallel walls
- Oval conidia









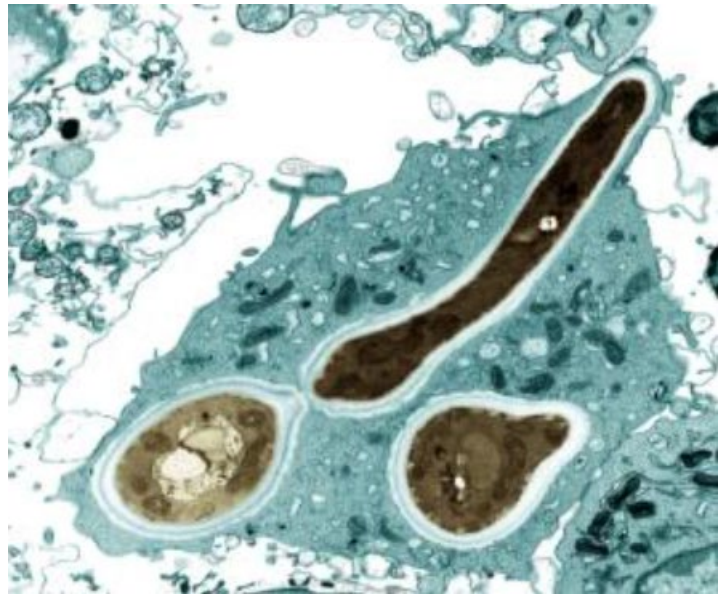
L. prolificans

- Annelides with swollen base and elongated neck
- Conidia are oval with narrow base

WHY IS *L. PROLIFICANS* DIFFERENT?

VIRULENCE FACTORS

- Can affect both immunocompromised and immunocompetent hosts
- After infection, transformation of conidia into hyphae
  - Formation of biofilms
  - Penetration of macrophages and tissues
    - *L. prolificans* can germinate within macrophages
  - Failure of host defence mechanisms
    - CNS macrophages respond poorly; reduced production of cytokines
      - Neurotrophism?
  - Melanin blocks phagolysosome formation



https://wellcomecollection.org/works/kmeyb3mh

Konsoula, A. et al. *Microorganisms* **2022**, 10, 1317.

CLINICAL MANAGEMENT
L. PROLIFICANS – ANTIFUNGAL SUSCEPTIBILITY (µg/µl)

	L. prolificans ($n = 14$)	*S. apiospermum* ($n = 48$)
Itraconazole	16/16 (16–16)	1/16 (0.25–16)
Voriconazole	8/16 (2–16)	0.5/1 (0.06–2)
Posaconazole	16/16 (16)	1/2 (0.25–2)
Amphotericin B	8/16 (8–16)	8/16 (1–16)
Caspofungin	4/8 (2–8)	1/2 (0.5–8)
Micafungin	4/8 (0.25–8)	0.25/1 (0.125–1)

[a]MIC50 and MIC90: MIC inhibiting 50% and 90% of the isolates tested, respectively.

Bronnimann D. et al. *Medical Mycology*; 59.2021:486–497

CLINICAL MANAGEMENT - AFST

- Resistance to all classes of antifungal agents
- Combination therapies - synergism
  - Voriconazole/terbinafine
  - Voriconazole/LAMB
- No *in vitro* activity to amphotericin B or echinocandins
- Voriconazole MIC 4μg/mL – may not be achievable *in vivo*
- Isavuconazole – No/limited activity; increased tolerability
- Reversal of neutropenia crucial
- Surgical debridement if possible

ECMM Guidelines:

Global guideline for the diagnosis and management of rare mould infections: an initiative of the European Confederation of Medical Mycology in cooperation with the International Society for Human and Animal Mycology and the American Society for Microbiology

- Hoenigl M. et al. *Lancet Infect Dis* 2021; 21: e246–57

COMMUNICATION OF LAB FINDINGS

- Notify clinical service of “septate hyphae” in BAL – Critical Value
- Lab should speciate *L. prolificans* vs. *S. apiospermium*
- Notification of final identification ASAP
- Educate on significance of antifungal susceptibility
- Encourage Infectious Disease consult

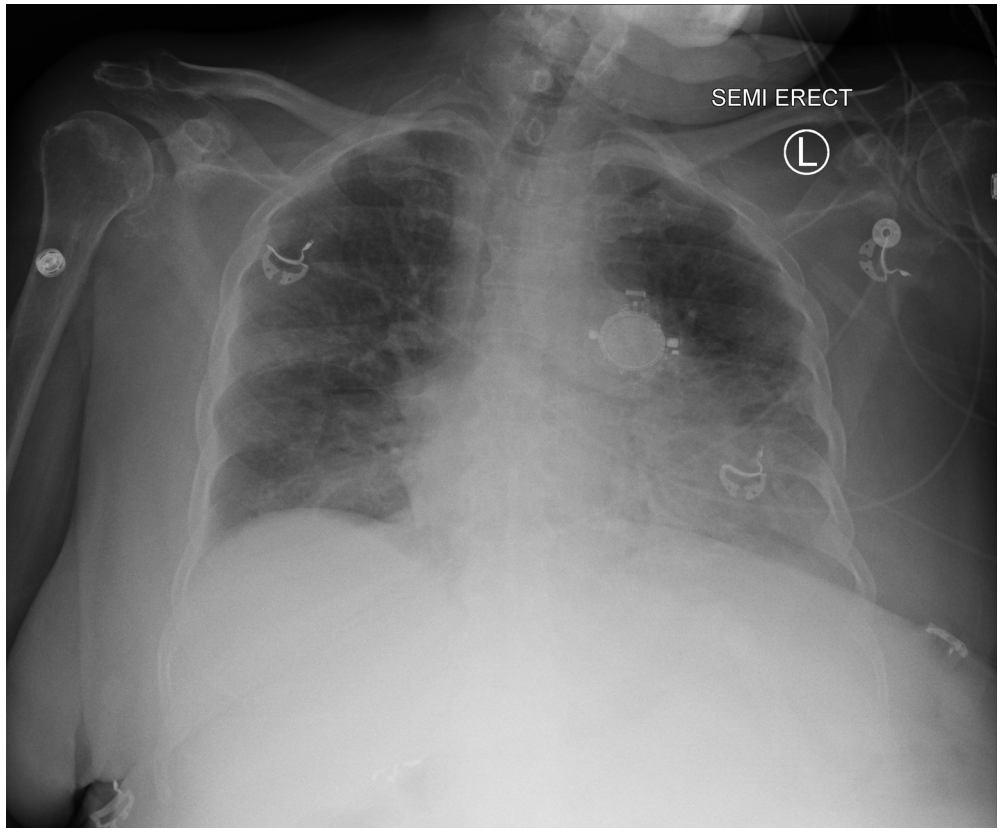
CASE #2

WHAT LIES BENEATH…

- 71 y.o. female
- Multiple strokes, altered mentation, DM
- Hospitalization 9/2021 – 10/2021 at OSH
  COVID pneumonia; tracheostomy
- Multiple transfers between OSH, long-term care and MCF
- MDR UTI due to *E. coli*
- *S. homnis* decubitus ulcer and osteomyelitis
- MRSA pneumonia
- Septic presentation; respiratory failure, admission to MCF 12/31/21

LABORATORY DIAGNOSTICS

- Temperature 37^{o}C, HR 74, BP 90/56
- WBC 11.2 X10^{9}/L
- Radiographs indicate areas of organizing pneumonia
  - Vancomycin, levofloxacin, aztreonam,
- 1/7/22: *Nakaseomyces glabrata* in urine
- 1/13/22: BAL positive for “yeast not *Cryptococcus* sp.” (not speciated)
- 1/31/22: MRSA in BAL
- 2/1/22: MRSA and “predominant yeast” in BAL (culture)



Pulmonary edema, decreased lung volumes

ANY OTHER CONCERNS?

2 DIAGNOSIS:

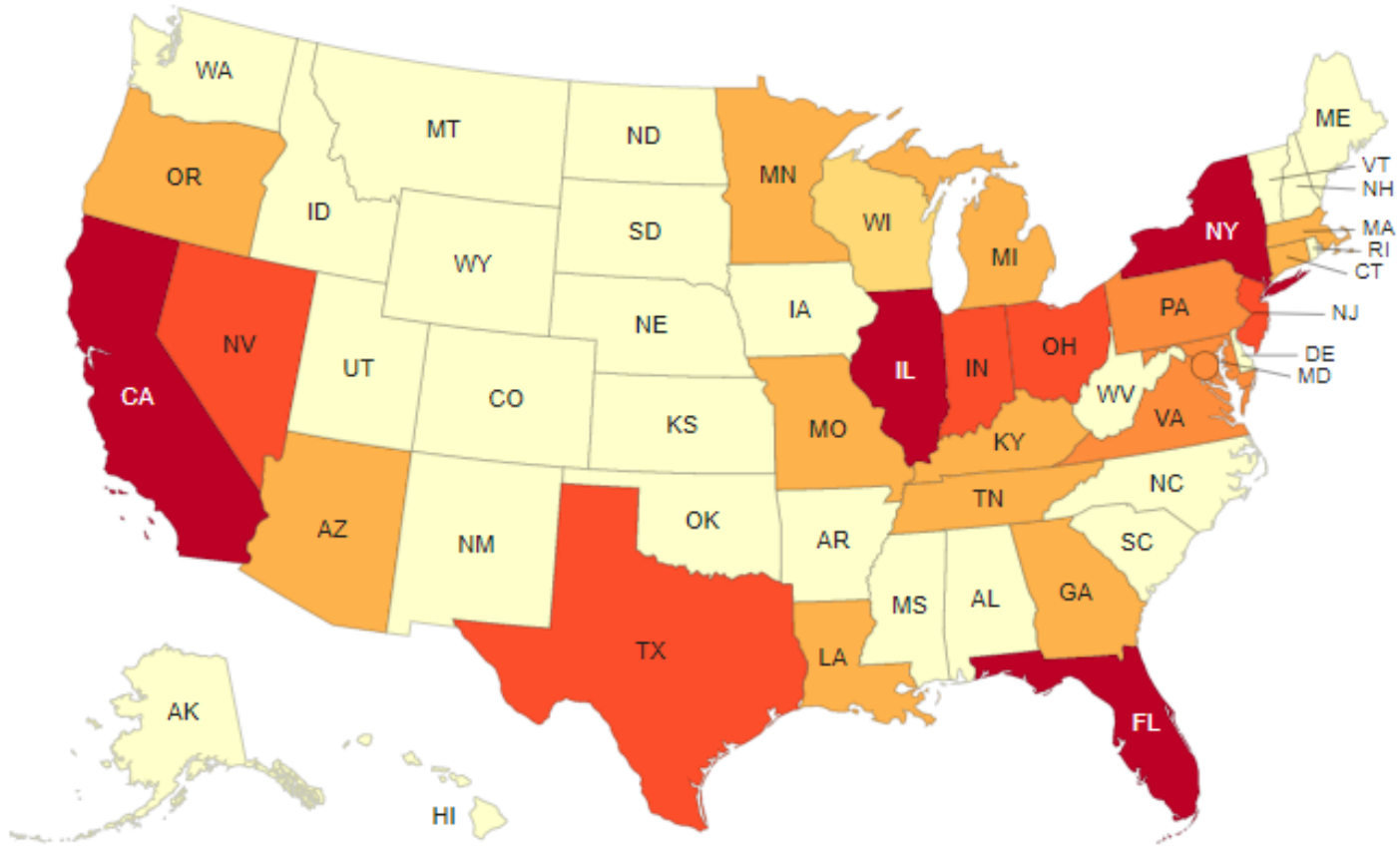
BAL COLONIZATION DUE TO *Candida auris*

GLOBAL EMERGENCE OF *CANDIDA AURIS*

- Japan 2009; Novel yeast isolated from ear
  - 85-87% sequence similarity to *C. haemulonii/C. pseudohaemulonii*
  - Not seen in culture collections prior to 2009 (SENTRY)
  - Noted on 5 continents less than 10 years after first reported
  - 4 clades: East Asia, South Asia, Africa, South America
  - Spread from major epicenters
    - Regional clonal expansion of highly related isolates
  - Risk from travel for medical reasons?
    - Germany 2015-2017
    - Canada 2017
  - Fell off the radar during COVID?

Hata, DJ et al. *Arch Pathol Lab Med.* 2019 Jun 6.
Schwartz, IS. *Can Commun Dis Rep.* 2017 Jul 6; 43(7-8): 150–153.
Hamprecht, A. et al. *Emerg Inf Dis.* 2019. 25;9:1763-1765.

Reported clinical cases of *Candida auris*, June 1, 2021-May 31, 2022



Number of Clinical Cases

- No cases reported
- 0 clinical cases, but ≥1 screening case
- 1 to 10
- 11 to 50
- 51 to 100
- 101 to 500

Territories: AS GU PR VI MP

https://www.cdc.gov/fungal/candida-auris/tracking-c-auris.html

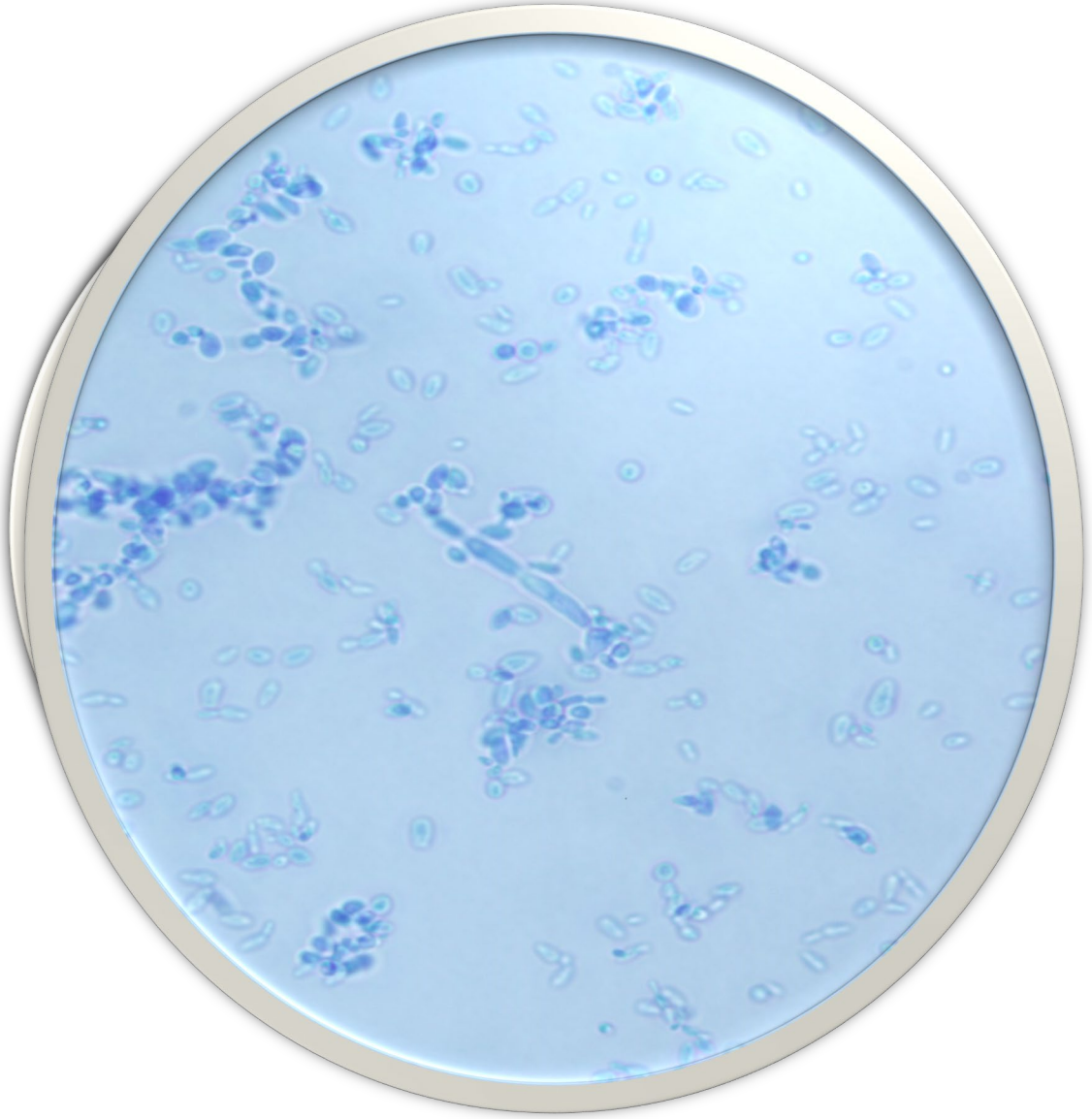
CLINICAL PRESENTATIONS ASSOCIATED WITH *C. AURIS* INFECTIONS

- Fungemia
  - 2012-2017: 742 isolates; 67% from blood
  - Mortality 28% - 60%
- CNS, bone, wounds, peritoneal, pericardial
- Transplant-associated
  - Bone marrow, lung
- Association with healthcare
  - Indwelling catheters
  - Broad-spectrum antimicrobial agents
  - ICU care; ventilators
  - Chronic conditions

Azar MM. *Clin Inf Dis*. 2017;65(6):1040–2
Vallabhaneni S et al. *MMWR*. November 11, 2016/65(44);1234–1237.

LABORATORY IDENTIFICATION OF *C. AURIS*

- Macroscopic
  - Moist, creamy colonies
  - Growth at 37 – 40° C on standard media
    - Sabouraud dextrose, brain heart infusion, inhibitory mold agar
  - Inhibition on media containing cycloheximide
    - SABHI, Mycosel
  - Challenges with chromogenic agar
- Microscopic
  - Oval to elongate – Similar to *N. glabrata*
  - Pseudohyphae and hyphae rare
  - Cannot be identified by morphology alone



Picture courtesy Diana Meza Villegas SM(ASCP)

LABORATORY IDENTIFICATION OF *C. AURIS*

- Commercially available automated phenotypic systems will mis-identify *C. auris*
  - BioMerieux Vitek, Becton Dickinson Phoenix, Beckman Coulter Microscan
  - Results of “no identification” or *Candida* sp.
  - Mis-identification
    - Results of C*. hameulonii, C. duobushhameulonii, Rhodotorula* sp.*, S. cerevisiae*
- Conventional biochemical methods not acceptable
- Chromogenic agar color can be variable

https://www.cdc.gov/fungal/candida-auris/identification.html

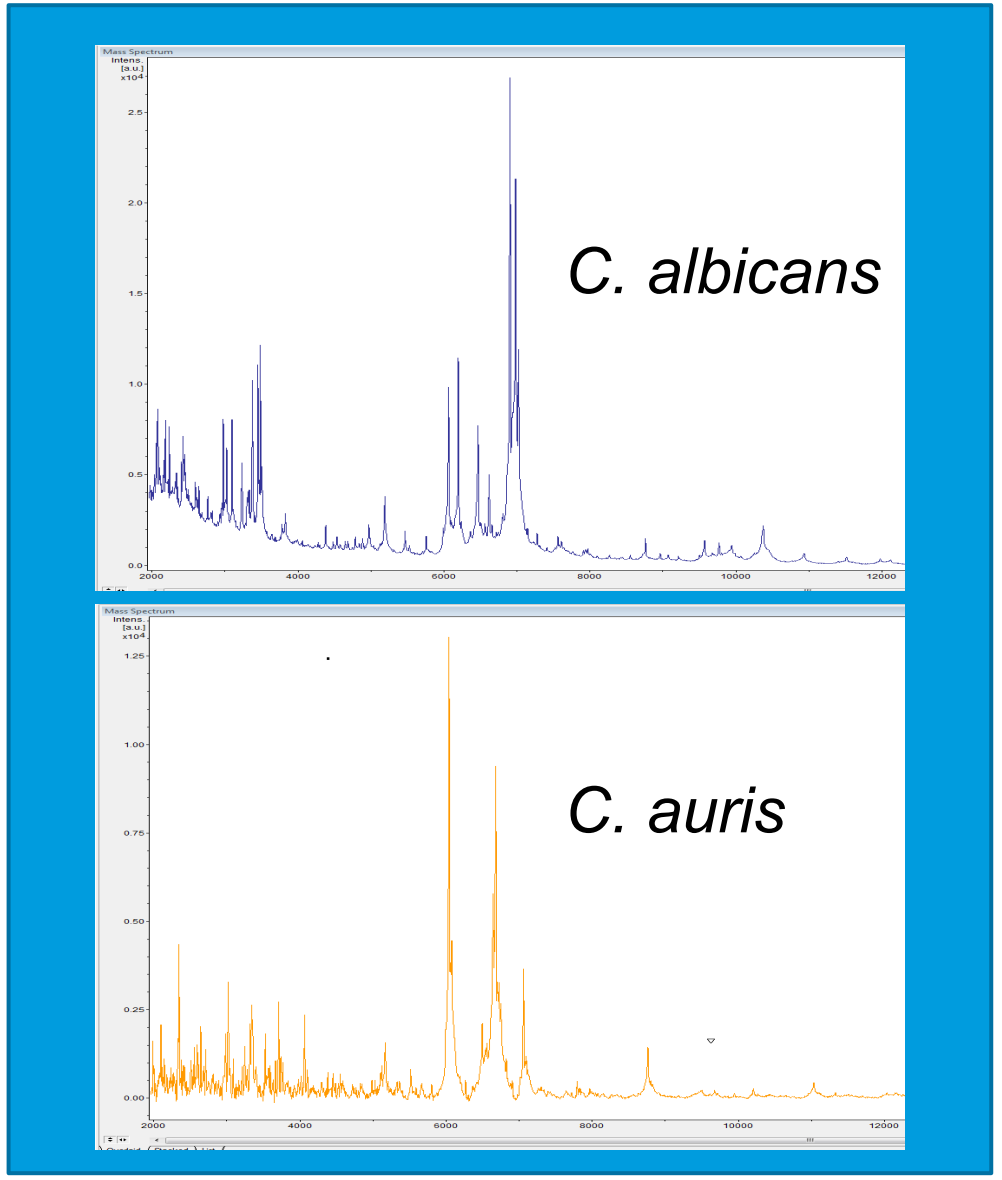
LABORATORY IDENTIFICATION OF *C. AURIS*

- Conventional Sequencing
  - ITS, D1 - D2 region of 28s rDNA
- Whole-genome sequencing (WGS)
  - Strain typing
- Molecular methods
  - PCR for detection and differentiation
- Magnetic resonance (T2 Cauris – RUO)
  - Direct from blood, skin samples
- Inclusion on blood culture panels – Check your panel!

Hata, DJ et al. *Arch Pathol Lab Med.* 2019 Jun 6.
Sexton DJ et al. *Mycoses*. 2018;61:786 –790.

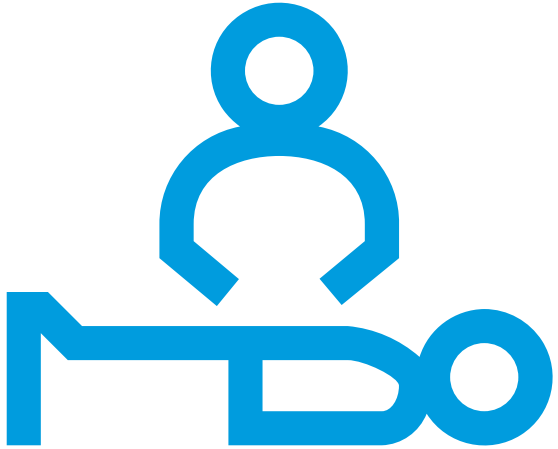
MALDI-TOF FOR *C. AURIS*

- Identification based on mass differentiation of ribosomal proteins
- Acceptable for confirmatory identification
- *C. auris* now included in instrument databases
  - Laboratory may develop own *C. auris* database
- Must start with pure culture (confirm!)
- Score of > 2.0 indicates identification to species
  - Our patient: 2.17



WHY IS *C. AURIS* DIFFERENT?

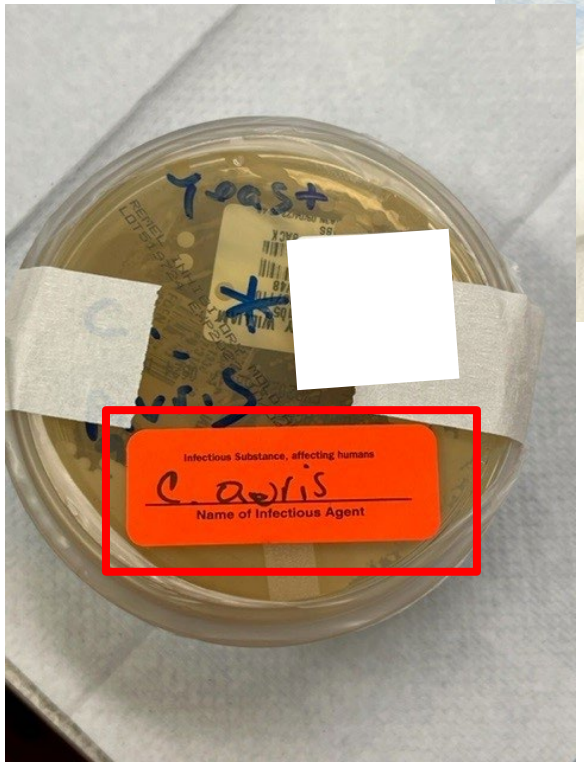
- Antifungal resistance profile
  - Reduced susceptibility to azoles, echinocandins, Amphotericin B
- Environmental persistence and viability
  - Dry cloth 7 days
  - Plastic surfaces 14 days
- Resistance to disinfection agents
  - Quaternary ammonium, acetic acid compounds NOT effective
- Ability to colonize patients
  - Hands, axilla, groin



Biswal M et al.2017. *Jour Hosp Inf*. 97:363-370.
Welsh RM et al. 2017. *Jour Clin Microbiol*. 55: 2996-3005.

LABORATORY SAFETY

- Manipulate *C. auris* in a biosafety cabinet
  - Yeast proven not to be *C. auris* may be handled on the open bench
- Wear appropriate personal protective equipment
  - Lab coat and gloves
- Disinfect with 10% bleach for 10 minutes
  - Follow with 70% ethanol rinse
- Label culture plates
- Notify laboratory staff



CLINICAL MANAGEMENT: TREATMENT OF *C. AURIS* INFECTIONS

- Accurate identification will guide anti-fungal selection
- Resistance to fluconazole (90%)
- Echinocandins considered first line therapy
  - Caspofungin, Micafungin, Anidulafungin
  - Amphotericin B for echinocandin failure
- Treatment of *C. auris* from non invasive sites, or if no evidence of infection is NOT recommended

Lockhart SR, et al. 2017. *Clin Inf Dis*. Jan 15; 64(2): 134–140.

RESISTANCE PROFILE OF *C. AURIS*

- Breakpoints/Interpretations not established by EUCAST or CLSI

	Candida albicans		*Candida auris* - Tentative	
μg/mL	CLSI	EUCAST	CLSI-MIC	EUCAST- ECOFF
Fluconazole	≥ 8	≥ 4	≥ 32	NA
Voriconazole	≥ 1	≥ 0.25	NA	8
Isavuconazole	NA	NA	NA	0.25
Posaconazole	NA	≥ 0.064	NA	0.25
Amphotericin B	ECV of 2	≥ 1	≥ 2	NA
Anidulafungin	≥ 1	≥ 0.032	≥ 4	0.5
Micafungin	≥ 1	≥ 0.016	≥ 4	0.25
Caspofungin	≥ 1	NA	≥ 2	NA

CLSI. Performance Standards for Antifungal Susceptibility Testing of Yeasts. 1st ed. CLSI supplement M60. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
Arendrup MC, et al.2017.*AntimicrobAgentsChemother*.;61:e00485–517

INFECTION CONTROL CONSIDERATIONS

- History of possible exposure
  - Long-term care
  - Healthcare exposure
    - Colonization with MDRO
  - International healthcare exposure

- Contact precautions
  - Swab of axilla and groin to assess colonization
  - May be required indefinitely
  - No recommendations for patient de-colonization

Strict adherence to environmental cleaning

- Bleach-based disinfectants, hydrogen peroxide

CLINICAL MANAGEMENT

- The first *C. auris* case at MCF! (High visibility from Administration)
- We were in an Omicron surge – enhanced PPE and limited visitors
- No antifungal treatment recommended
  - Colonization, not infection
  - Treatment not recommended for non-invasive infection
  - https://www.cdc.gov/fungal/candida-auris/c-auris-treatment.html
- Supportive care for comorbidities
  - Clinically improving
- Modified contact precautions until discharge
- Notification of long-term care facility upon discharge
- Communication between lab, Infectious Disease service and Infection Prevention

INFECTION PREVENTION RESPONSE

- Point prevalence study of patient ward
- Swabbing of axilla and groin of patients in surrounding rooms
  - N=20
- Submittal to CDC AR Lab network (Tennessee)
- Swabs of environmental surfaces
  - Bedrails, TV remote, bed controls
- All swabs negative

REALLY?? IS NOTHING SACRED?

RESEARCH ARTICLE
March/April 2022 Volume 13 Issue 2 e00518-22
https://doi.org/10.1128/mbio.00518-22

Candida auris on Apples: Diversity and Clinical Significance

Anamika Yadav[a,b], Kusum Jain[a,b], Yue Wang[c], Kalpana Pawar[a], Hardeep Kaur[b], Krishan Kumar Sharma[d], Vandana Tripathy[d], Ashutosh Singh[a], Jianping Xu[c], Anuradha Chowdhary[a]

CASE #3 THE NEED FOR SPEED…

- 61 y.o. female
- HTN, hyperlipidemia, DM 2 months
- 8/9/2021: COVID pneumonia
  Dexamethasone
- 8/22/2021: Admitted to OSH (5 days)
  COVID positive, anxiety
  Dexamethasone, Hgb A1C 14% (<7% normal)
  (R) Eye pain; resolved with analgesics
- 8/26/2021: Discharged, returned 8/27 with acute eye pain. Referred to outside ophthalmologist for follow up
- 8/30/2021: Complete loss of vision
- 8/31/2021: Presented to MCF ED

ED PRESENTATION

- BP 124/74, HR 87, Temperature 37^0 C, Resp 18, SpO_2 94%
- Tenderness on frontal and maxillary region of (R) face
- Loss of vision, eye pain, ptosis
- CT: maxillary, ethmoid, sphenoid sinusitis. Inflammation surrounding optic nerve, extending to orbital matrix
- Right orbital decompression in OR

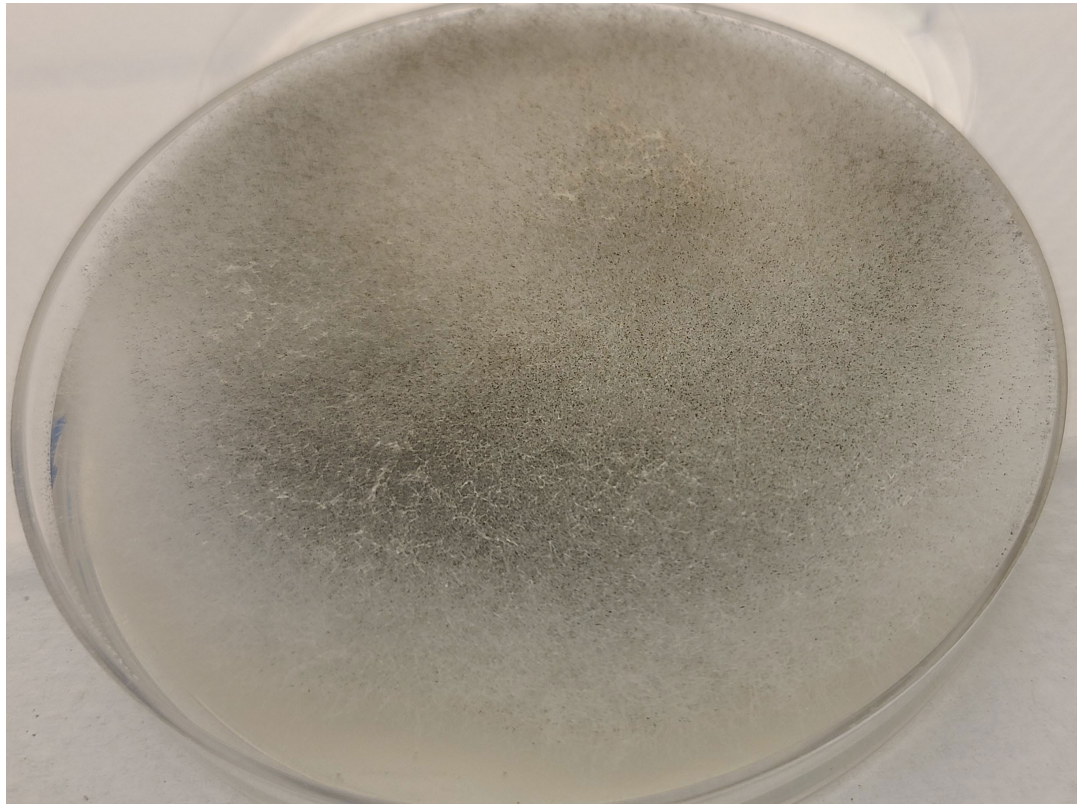
OR REPORT

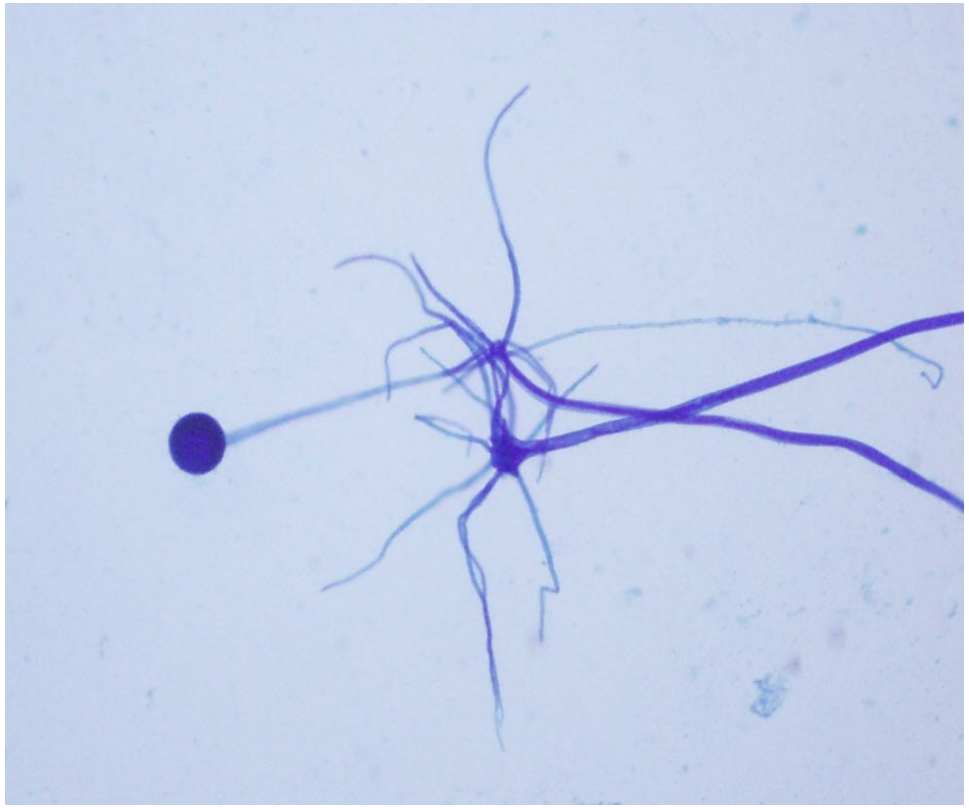
- Inferior turbinates was dusky and black
- Middle turbinate was black and necrotic
- Significant portions of the ethmoid sinuses were dusky and necrotic
- Diffuse necrosis of the lateral wall of the maxillary sinus
- Medial wall of the maxillary sinus was completely removed as it was necrotic
- Bone of the pterygopalatine fossa appeared viable
- Necrotic mucosa around the frontal recess. This was completely removed.
- Medial rectus [perioccular] muscle was dusky. The orbital fat was dusky
- Extensive resection of sinuses, decompression of optic nerve

TISSUE BIOPSY FROM SINUS

- GNB Reported 00:54 (3rd shift)
- Fungal smear negative
- Waiting for something to happen……

Fungal cultures reviewed/confirmed at 14 hours:





WHAT DO YOU THINK?

3

DIAGNOSIS:

RHINOCEREBRAL MUCORMYCOSIS DUE TO *RHIZOPUS ARRHIZUS* (POST COVID)

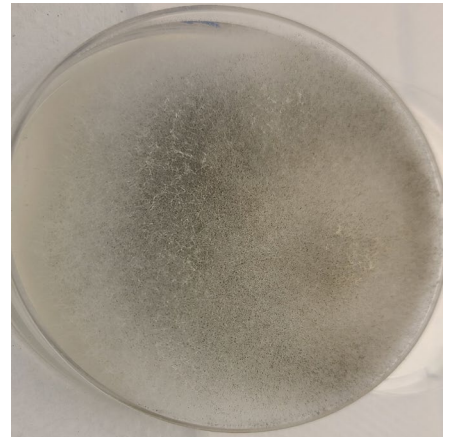
FUNGAL TAXONOMY

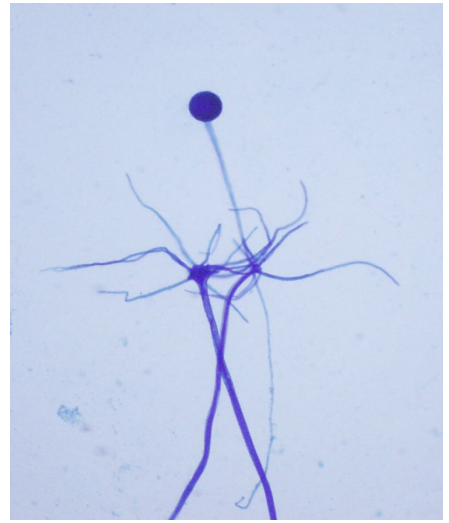
- Order *Mucorales*
  - *Zygomycetes* obsolete
  - *Rhizopus*, Mucor*, Lichtheimia* (Absidia),Rhizomucor Cunninghamella, Apophysomyces, Saksenaea, Syncephalastrum*
    - * Most often isolated from orbital sites
- Challenges in identification to species level
  - Growth conditions, morphology
  - ITS sequencing preferred
  - Not necessary for initial clinical management

Badali H. et al. 2021. *Jour Clin Microbiol*. 59:e0123021.

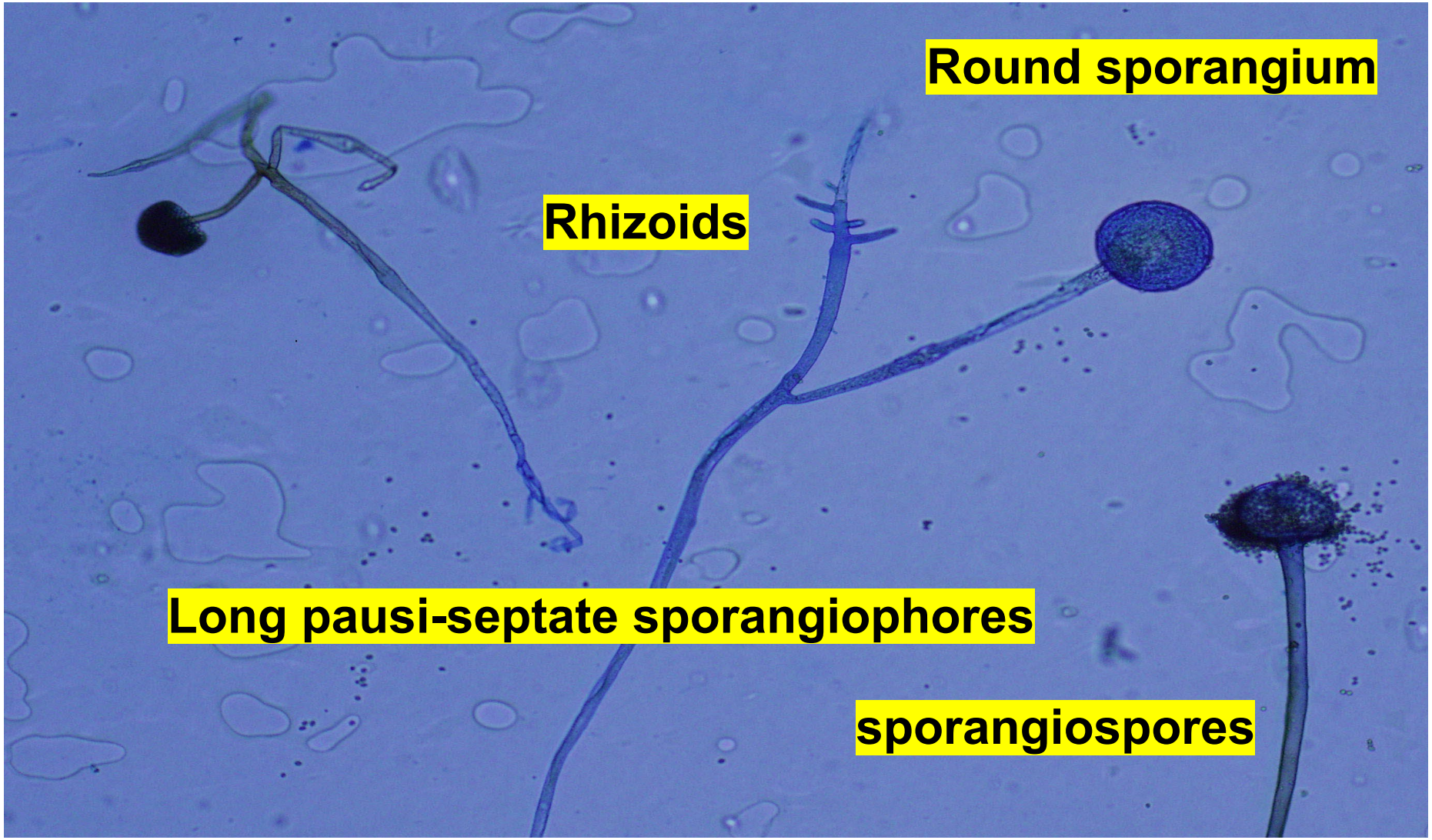
RHIZOPUS SP. – CHARACTERISTICS

- Rapid growth on standard fungal media at 30^0C
  - Inhibited by cycloheximide (Mycosel)
  - Lid lifters - tape plates shut!
- White fluffy colony turning grey to grey-brown
  - Reverse is white
- Long sporangiophores with round or football-like dark sporangia
- Rhizoids at the base of the sporangia
- Suppurative necrosis, occasionally granulomatious reactions
- Immunosuppressed hosts; angioinvasive

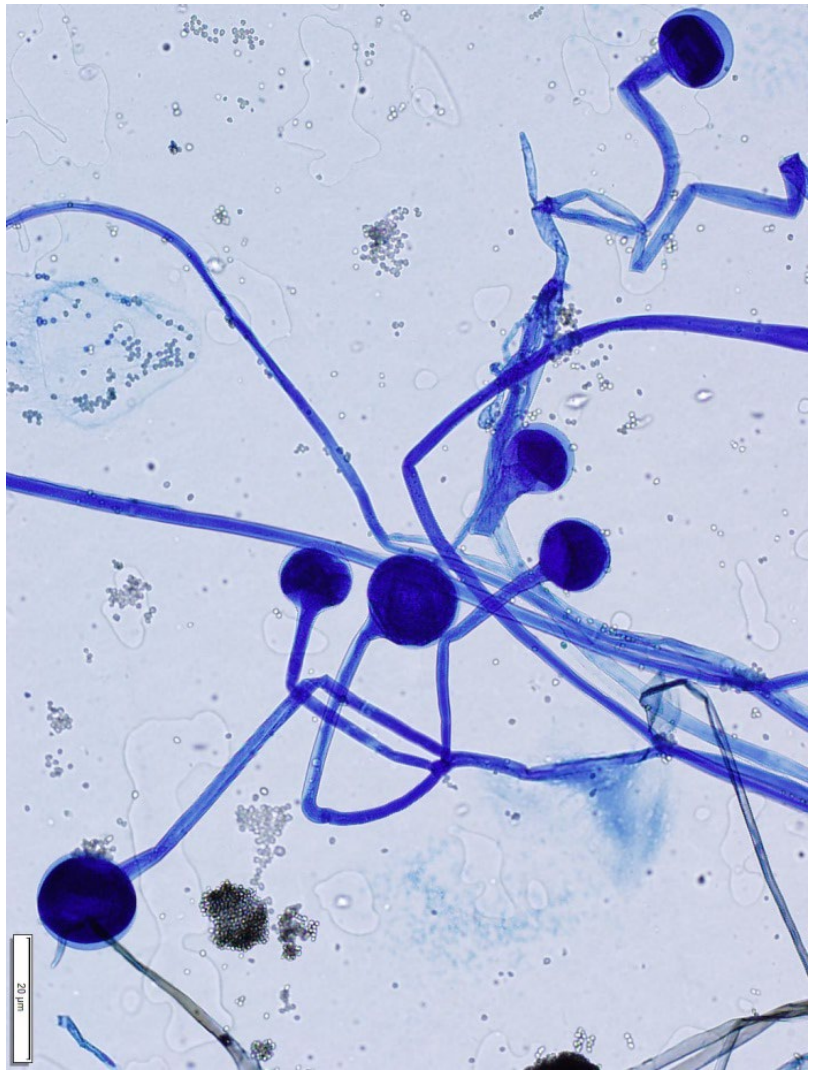




RHIZOPUS SP.



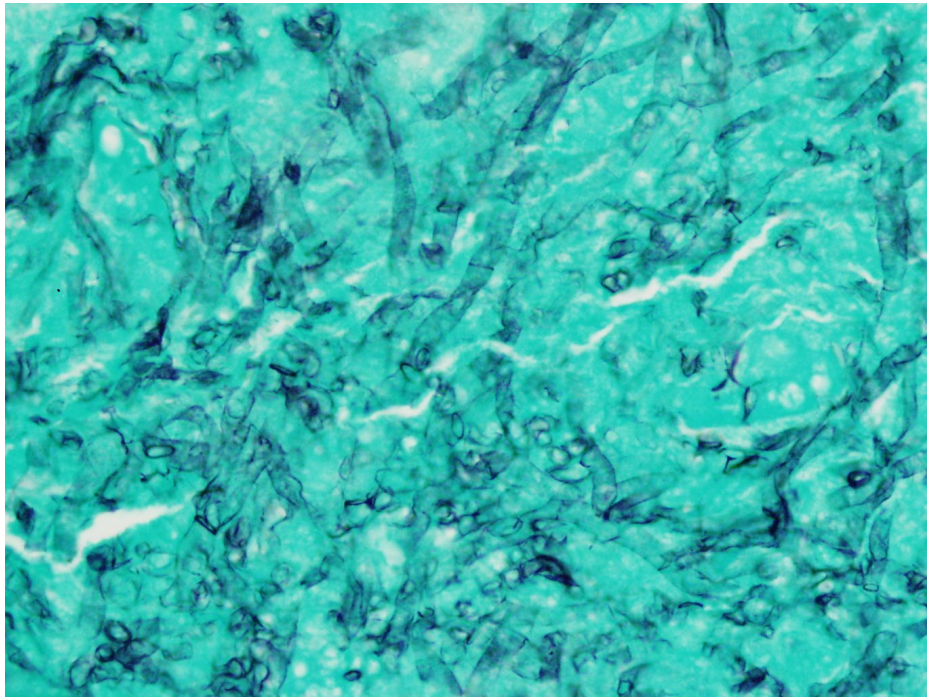
MCF Mycology Lab

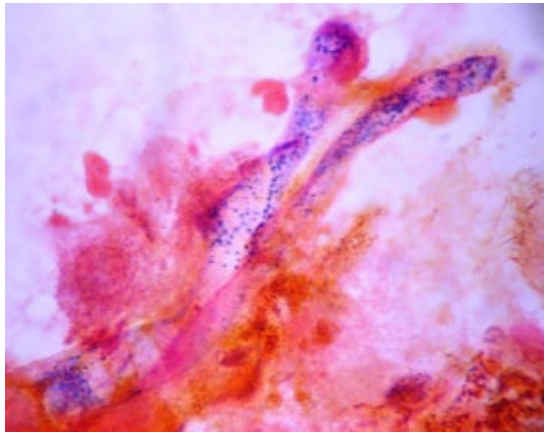


Broad ribbon-like pausi-septate hyaline hyphae
90^{o} branching may be difficult to see

LABORATORY DIAGNOSTICS

- Histopathology with HE, PAS, GMS
  - Necrosis, angioinvasion, neutrophilic infiltration
- Calcofluor white for direct smears
- Gram stain may be useful
- Culture confirmation required
  - Do not grind tissue
- Biomarkers **not** useful
  - Beta-glucan, galactomannan
- Molecular methods (PCR) not standardized, but may be useful
- Sequencing, MALDI-TOF generally not routine

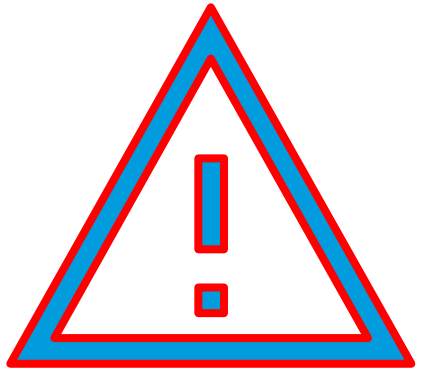


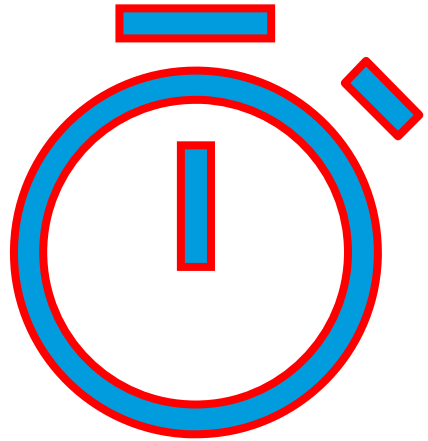


Cornely OA. Et al. 2019.*Lancet Inf Dis*.19:e405-e421.
Lau C et al. 2011. *The Neurologist* 17:151-153.

COMMUNICATION OF LAB FINDINGS

- Identification of *Mucorales* is a critical (panic) value, and should be immediately communicated
  - All technologists should be able to identify hyphae
- Make sure that providers understand
  - “Zygomycete -> Mucormycete”
- Identification to genus level is sufficient for treatment
- Educate on significance of antifungal susceptibility
  - Not needed for empiric therapy!
- Encourage urgent ophthalmology, laboratory, and infectious disease consult





RHIZOPUS SP. – SUSCEPTIBILITY

- Amphotericin B + surgical debridement recommended (empiric)

	Range μg/mL	Our case μg/mL
Amphotericin B	≤0.03 - 2	0.06
Posaconazole	≤0.03 - >16	0.5
Isavuconazole	0.125 - >16	8
Itraconazole	0.06 - >16	
Voriconazole	Inactive	
Echinocandins	Inactive	

AFST performed at UT San Antonio Fungal testing laboratory

- Combination therapy?
  - AMB + caspofungin or terbinafine
- Isavuconazole has similar MIC's as posaconazole
  - Approved for oral treatment of mucormycosis

Badali H. et al. 2021. *Jour Clin Microbiol*. 59:e0123021.
Cornely OA. Et al. 2019.*Lancet Inf Dis*.19:e405-e421.

“COVID ASSOCIATE MUCORMYCOSIS”

RHIZOPUS ARRHIZUS MOST COMMON

- “CAM” defined as infection concurrent or within 60 days of proven COVID
  - Mean duration 15 days after COVID diagnosis
- Global prevalence estimated at 0.70% (0.03 – 4.25%)
- Rhinocerebral form most common
- Mortality 29.6%, 75% DM, 95% steroid use for COVID
- Reduction in CD4 and CD8 T cells due to COVID - reduction in IL 4,10,17
- DM delays gamma-IF response
- Clinical awareness is essential!

Hussain S. et al.*J Fungi*.2021.7(11):985
Aranjani JM et al. 2021.*PLoS NeglTrop Dis* 15(11):e0009921
Revannavar SM et al. 2021. *BMJ Case Rep*. 14:e241663.

RESOLUTION OF CASE

- Remdesivir, LAMB, Vancomycin, Cefepime, Metronidazole
- Respiratory failure, COVID pneumonitis
- Sinusitis with MRSA, pan-susceptible *P. aeruginosa*
- Discharged 9/10/2021
- Follow up 4/2022: post-surgical changes, thickening of soft tissue and mucosa of sinuses. Complete loss of vision in (R) eye
- Plan to continue isavuconazole 6 – 12 months

CASE #4

DOUBLE THE TROUBLE

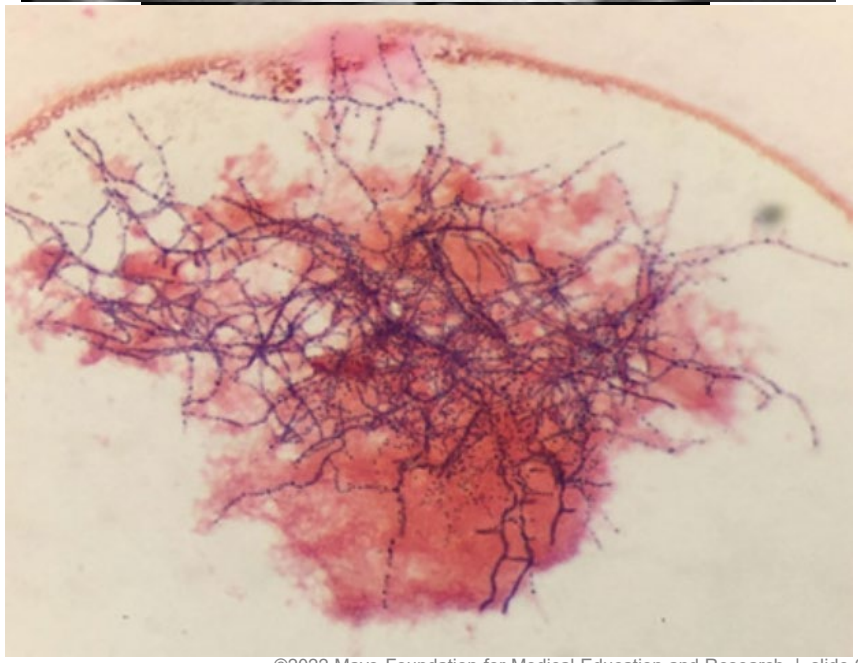
- 34 y.o. male
- ESKD due to hypertensive nephrosclerosis
- 2015 renal transplant. CMV D (+), R (-)
  - CMV nephritis at 1-year post-transplant
- Tacrolimus, mycophenolate, prednisone
- 2018 *Nocardia farcinica* L elbow and shoulder (OSH) – Linezolid and doxycycline
- January 2019 Fluid collection at graft site requiring aspiration
  - Placed on isavuconazole for “fungus”
- Return to MCF March 2019 for opinion on management
  - RLQ pain and 13 kg weight loss

LABORATORY DIAGNOSTICS

- Hemoglobin 6.9 g/dl
- WBC 3 x10^9/L
- Platelets 203 X10^9/L
- Creatinine 6.24 mg/dl
- BK and CMV PCR negative, HIV p24 and Aby negative
- CT guided aspiration of kidney; drain placed

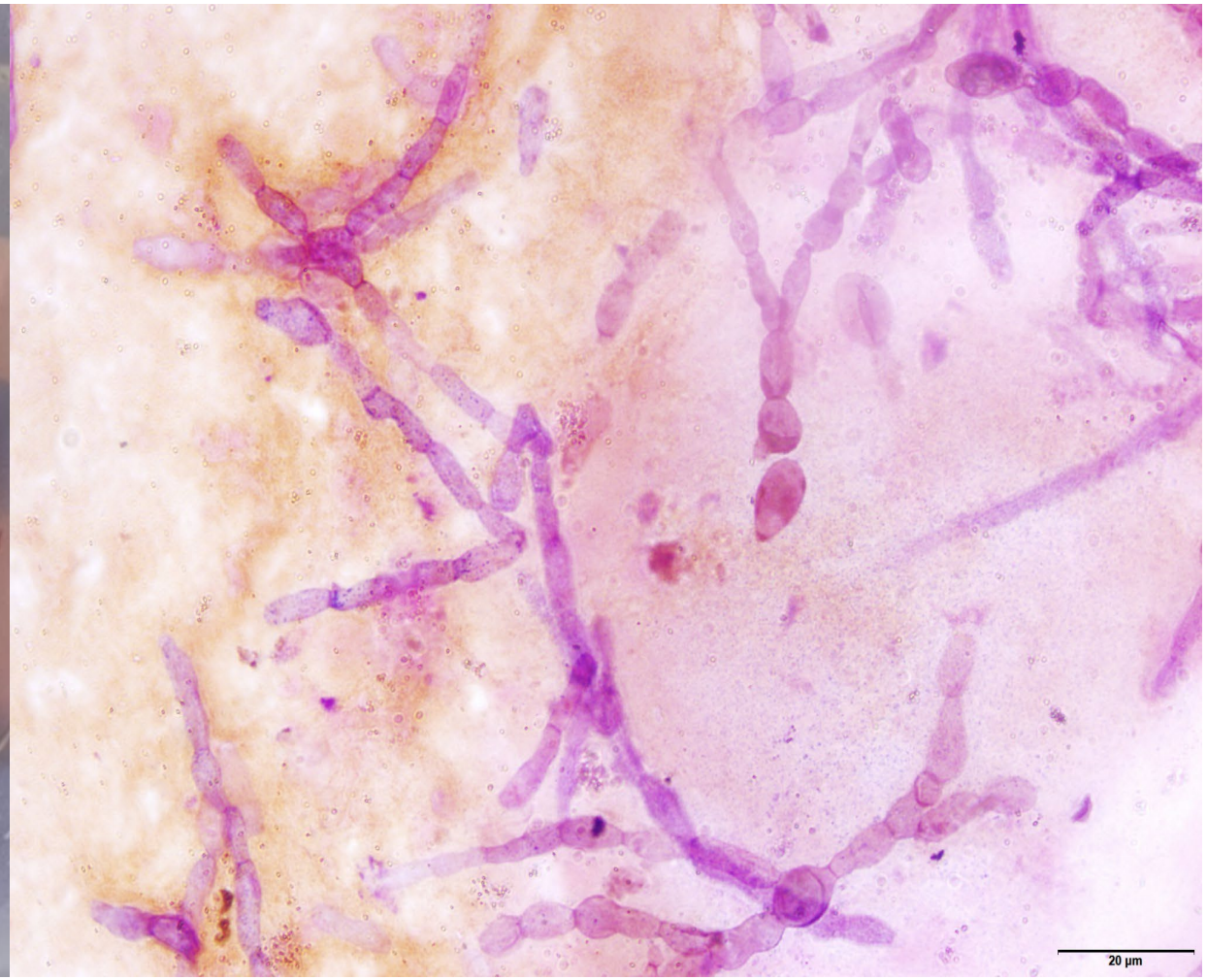
HOSPITAL COURSE

- Acute epistaxis, disclosed he had been coughing for several weeks
- CT of chest, brain and sinuses
  - Ground glass opacities in LLL
  - IV TMP/SMZ, meropenem
  - Craniotomy with drainage of abscess
  - Specimen submitted for culture
    - Organism isolated
- Nephrectomy with abscess drainage and debridement of muscle
  - Specimens submitted for culture



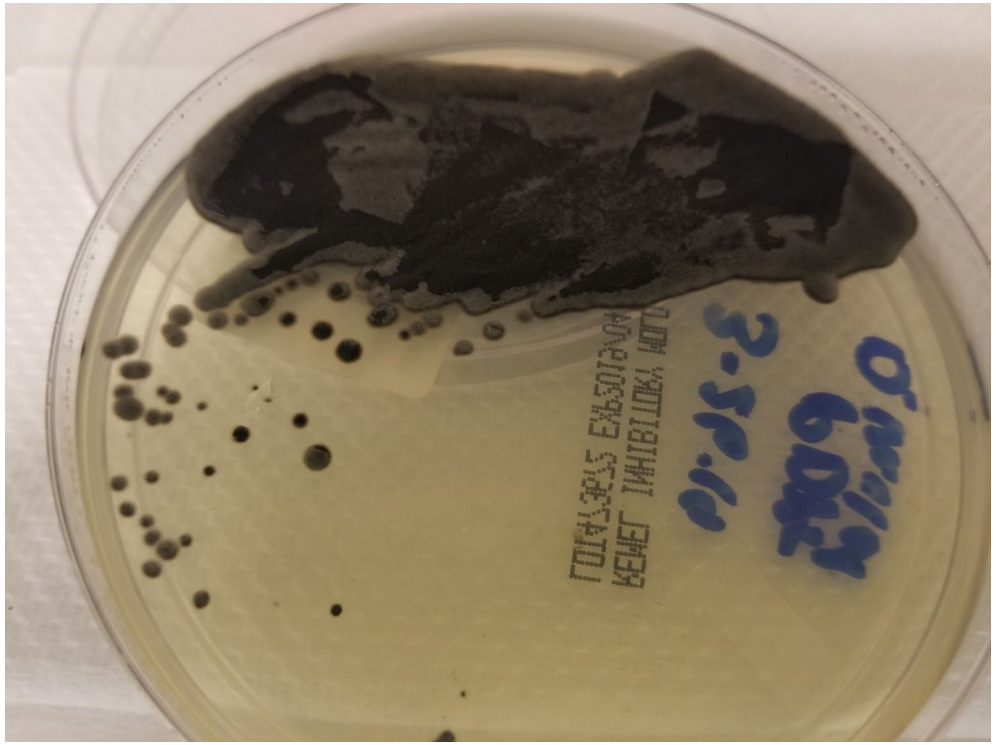
Cortes P et al. *Immun Inflamm Dis*. 2021 Dec;9(4):1146-1152.

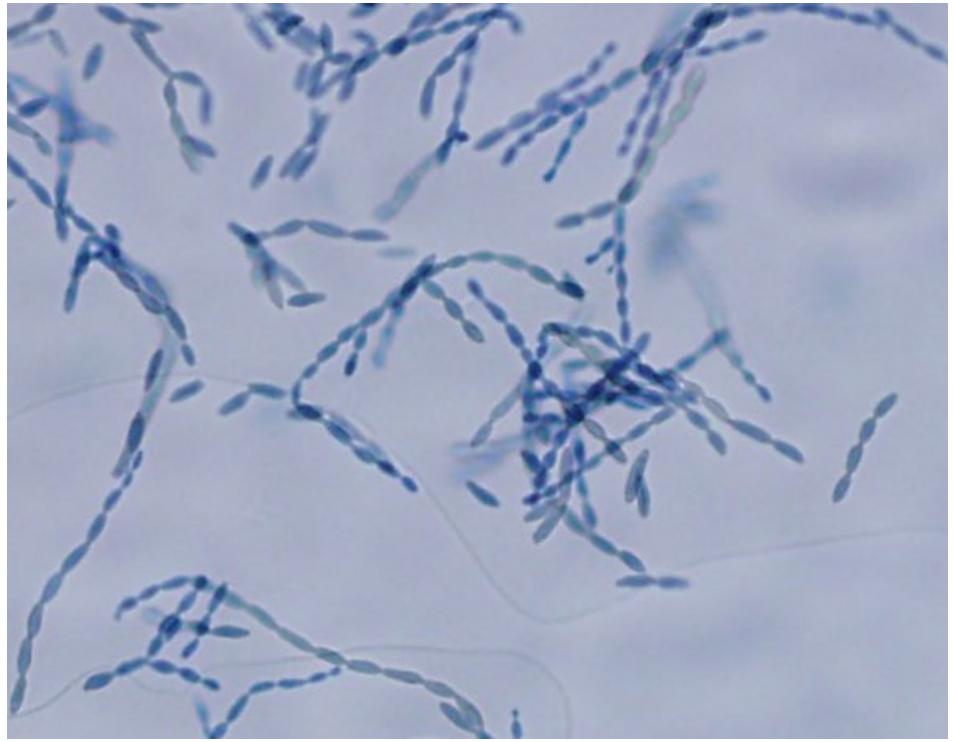
SPECIMEN RECEIVED IN MICROBIOLOGY



FUNGAL CULTURE RESULTS

- Gram stain and fungal smear positive
  - Culture of tissue positive for dematiaceous fungi at 7 days. Final ID at 16 days





WHAT DO YOU THINK?

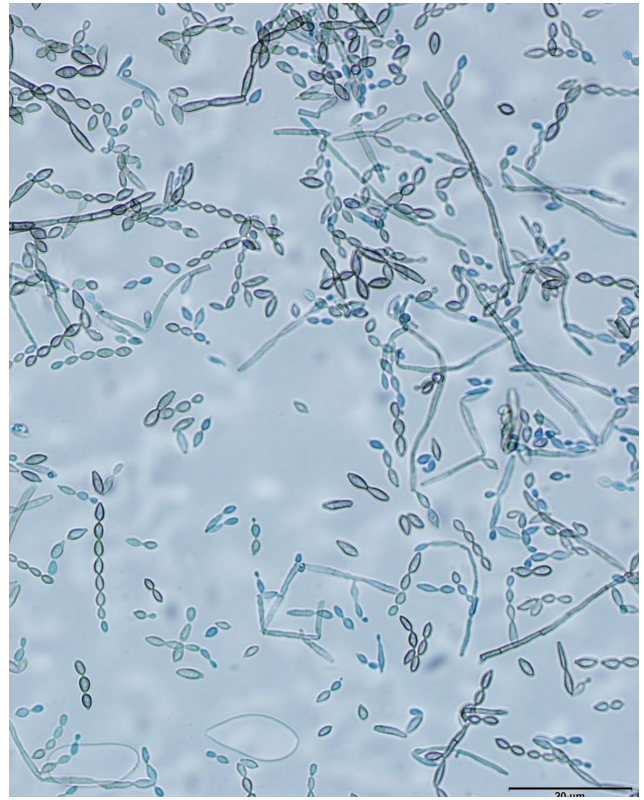
DIAGNOSIS:

4

RENAL ALLOGRAPH INFECTION DUE TO *CLADOPHIALOPHORA BANTIANA* WITH CNS *NOCARDIA FARCINCA*

CLADOPHIALOPHORA BANTIANA

- Formerly:
  - *Cladosporium bantianum*
  - *Cladosporium trichoides*
  - *Xylohypha bantiana*
- Dematiaceous fungi (melanin)
- Slow grower (15 d), can tolerate 42° C
- Long chains of sparsely branched septate hyphae
- Oval conidia
- Neurotrophic
  - Handle cultures carefully in the laboratory – no slide cultures!



Garzoni C et al. 2008. *Medical Mycology;*46:481-486.
Larone, D. H., et al. (2018). *Larone's Medically Important Fungi*. Washington, DC, ASM Press.

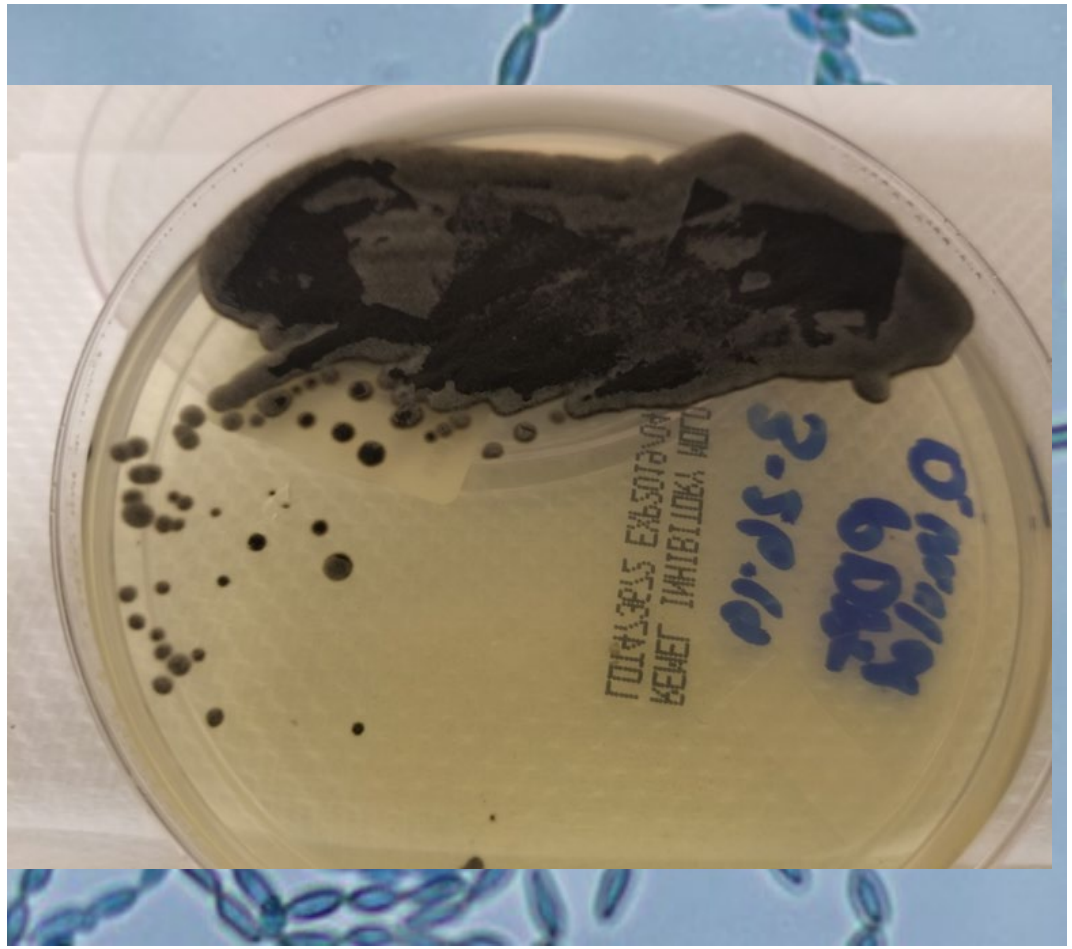
CLADOPHIALOPHORA BANTIANA

EPIDEMIOLOGY

- Saprophytic; worldwide distribution
- 50% of brain abscesses due to dematiaceous fungi (melanin)
- Associated with higher mortality in immunosuppressed patients (77%)
- Respiratory exposure, traumatic inoculation
- Hematogenous dissemination to CNS
- Phaeohyphomycosis
  - Brain abscesses – single and multiple lesions; delayed diagnosis
  - Soft tissue infections, sinusitis, mycetoma

Chakrabarti A et al. 2016. *Medical Mycology*;54:111-119

C. CARRIONII VS *C. BANTIANA*



C. BANTIANA AND *C. CARRIONII*

MORPHOLOGY DIFFERENCES

- ID in this case based on morphology, ITS sequencing
- Plasma metagenomic NGS in pediatric case

Organism	Conidiophores	Shield Cells	Conidia Shape	Hila on Conidia	Conidia Chain Length	Conidial Branching	Growth Temperature C°
C. carrionii	+/-	+/-	Oval	+/-	Medium	Moderate	35 - 37
C. bantiana	-	-	Oval	-	Long	Sparse	42 - 43

Adapted from: Larone, D. H., et al. (2018). *Larone's Medically Important Fungi*. Washington, DC, ASM Press.
Boguniewicz, J et al. *The Pediatric Infectious Disease Journal*: August 31, 2022 - Volume - Issue - 10.1097

ANTIFUNGAL SUSCEPTIBILITY TESTING

- No established AFST breakpoints
- Limited data (*in vitro*)

Drug	*Cladophialophora bantiana* (n = 37)			
	Range	Geometric mean	50%	90%
Amphotericin B	0.125–2	0.7	1	1
Fluconazole	16–64	35.14	32	64
Itraconazole	<0.016–0.25	0.064	0.063	0.125
Voriconazole	0.125–4	0.769	1	2
Posaconazole	<0.016–0.25	0.044	0.031	0.125
Isavuconazole	0.008–1	0.259	0.25	0.5
Caspofungin[a]	1–8	2.551	2	4
Anidulafungin[a]	0.016–4	0.073	0.063	2

Badali H et al. *J Clin Microbiol*. 2010 Jul;48(7):2350-6.

Recommendations

- Neurotrophic isolates
  - Itraconazole
  - Posaconazole
  - Isavuconazole
  - Voriconazole
- No synergism
- AMB, echinocandins have low CNS penetration

CLINICAL MANAGEMENT

- Review of 124 culture proven *C. bantiana* cases
  - Overall mortality 65.2%
- Surgical excision + LAMB
  - 54% survival when complete excision possible
- No standard therapies recommended
  - Excision + therapy
- ECMM 2021 guidelines
  - Moderate recommendation for voriconazole or posaconazole
  - May add echinocandins, LAMB

Chakrabarti A et al. 2016. *Medical Mycology*; 54:111-119
Hoenigl M. et al. *Lancet Infect Dis* 2021; 21: e246–57

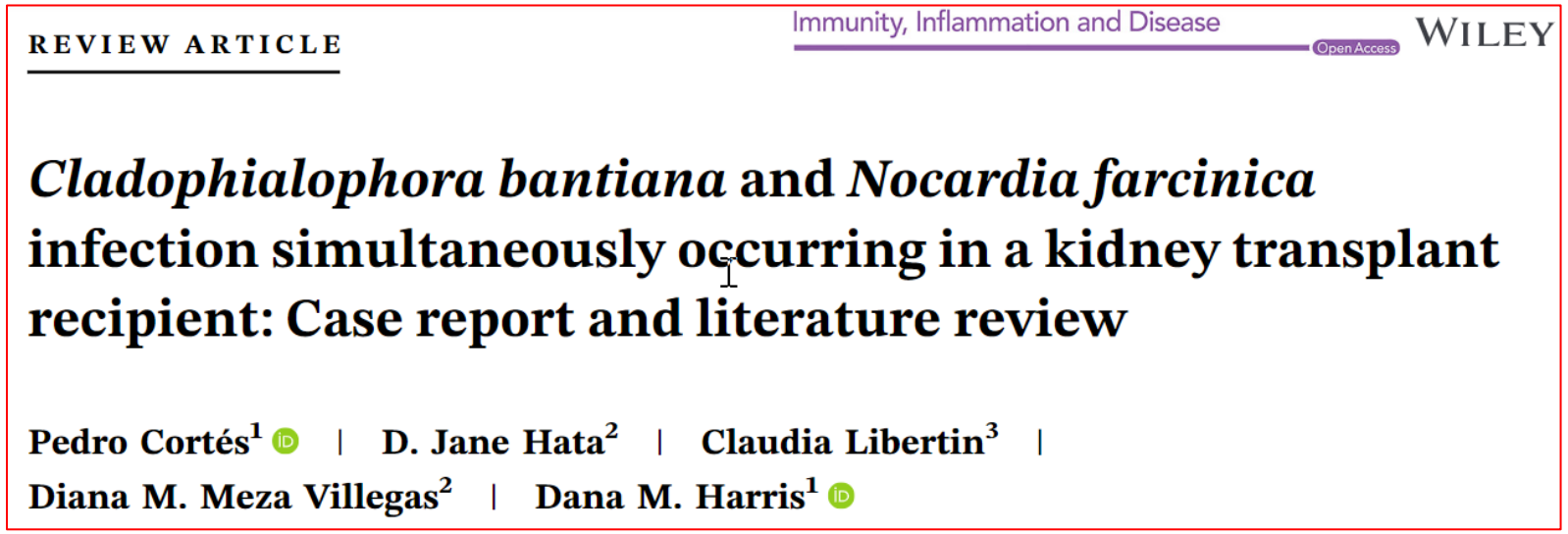
LABORATORY CASE MANAGEMENT

- Maintain a high level of suspicion when dealing with dematiaceous fungi
- ALWAYS think biosafety!
  - *C. bantiana* affects both immunocompromised and immunocompetent
  - No occupational infections reported, but subcutaneous inoculation or inhalation exposures possible
  - BSL-2, but handle in BSC
- Notification of final identification ASAP
- Encourage Infectious Disease consult

BMBL 6th ed. 2020.

RESOLUTION OF CASE

- Renal function did not improve
  - Initiate hemodialysis
- No CNS dissemination
- Discharged after 14 days
  - LAMB, voriconazole, meropenem, TMP/SMZ
- Repeat imaging planned in 6 weeks
- Patient lost to follow up



REVIEW ARTICLE

Immunity, Inflammation and Disease Open Access WILEY

***Cladophialophora bantiana* and *Nocardia farcinica* infection simultaneously occurring in a kidney transplant recipient: Case report and literature review**

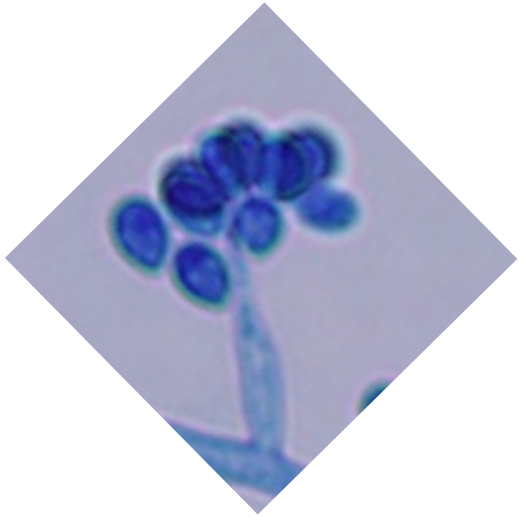
Pedro Cortés[1] | D. Jane Hata[2] | Claudia Libertin[3] | Diana M. Meza Villegas[2] | Dana M. Harris[1]

Cortes P et al. *Immun Inflamm Dis*. 2021 Dec;9(4):1146-1152.

SUMMARY AND RECOMMENDATIONS

SUMMARY

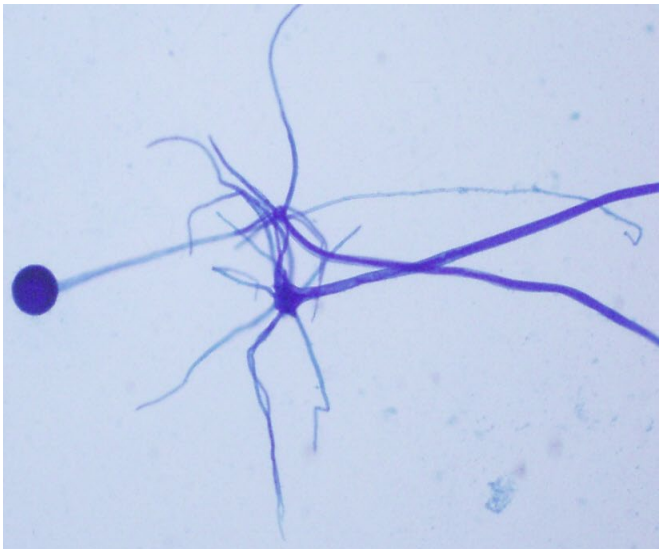
- Diagnostic Methods
  - Many new technologies, but culture is still gold standard
- *Lomentospora prolificans*
  - Flask-shaped conidiophores with swollen bases
  - Important to differentiate from *S. apiospermium*
  - Resistance to all classes of antifungal agents
- *Candida auris*
  - Fungemia, CNS, lung, peritoneal
  - Challenges to identification
  - ~~Fluconazole~~
  - *Healthcare associated; environmental persistence*
  - Infection Control considerations

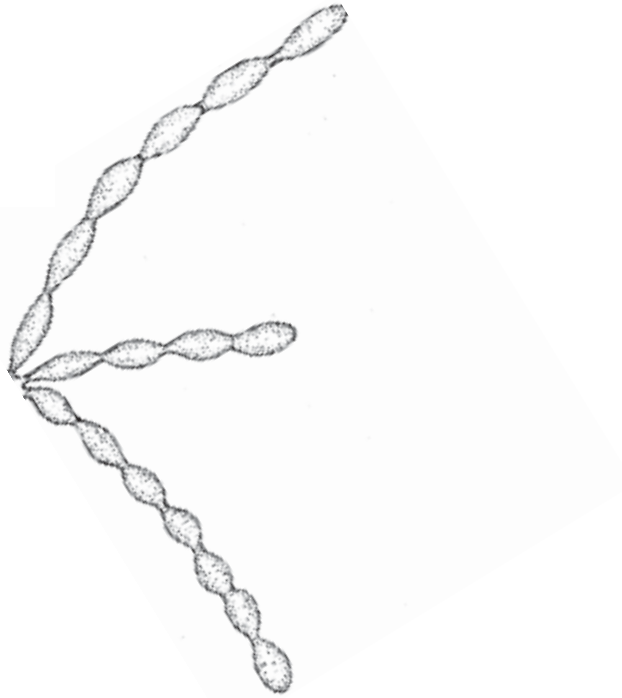


SUMMARY

- *Mucorales – Rhizopus arrhizus*
  - Tissue destruction; Angioinvasive
  - Grows fast *in vitro* – Check plates often!
  - Identify *Mucorales* and communicate to service
  - Debridement and AMB
- *Cladophialophora bantiana*
  - Dematiaceous
  - Long chains of conidia
  - Neurotrophic
  - Biohazard – work carefully!

- **Always ensure good communications between the lab and clinical services**





THANK YOU FOR YOUR ATTENTION!

QUESTIONS & ANSWERS

MCF Mycology All Stars: Randy Rouse, Diana Meza-Villegas, Lauren Frank and Fred Westenfeld!

