Diagnostic Mycology for Laboratory Professionals
Part One--Yeast

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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. Medical mycology basics

   A. Comparisons with other life
   B. Classification

II. Identification of clinically-significant yeast

   A. Cell morphology hints
   B. Cultivation hints
   C. Rapid and definitive methods

III. Antifungal susceptibility testing

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

The Basics

KINGDOM DIFFERENTIATION

Characteristic	Fungi	Plantae	Bacteria†	Animalia
Eukaryote	Yes	Yes	No	Yes
Membrane-bound organelles	Yes	Yes	No	Yes
Non-mitochondrial ribosomes	80S	80S	70S	80S
Chlorophyll	None	Yes	Some	No
Obligate anaerobes	None	None	Some	No
Specialized tissue	Sort of	Higher plants	No	Yes
Sterols in membrane	Yes	Yes	*Mycoplasma*	Yes
Cell wall	Chitin, mannan, glucan, chitosan, glycoprotein	Cellulose	Peptidoglycan	No

en.wikipedia.org

†Six-kingdom classification consists of either two Bacteria kingdoms or two Protista kingdoms

SCOPE OF FUNGI

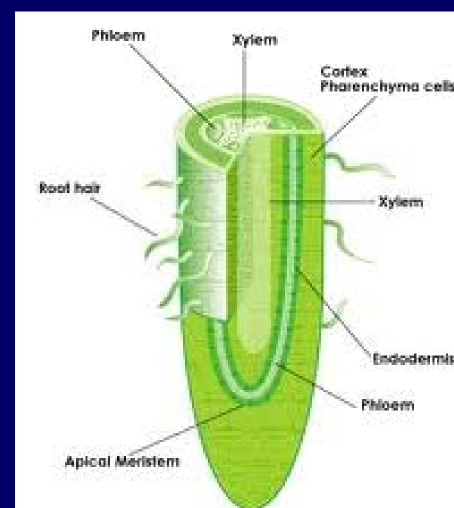
- At least 100,000 named fungal species
- ~1 million to 10 million unnamed species; 1000 to 1500 new species per year
- Fewer than 500 named species associated with animal or human disease
- Less than 50 are pathogenic in healthy human hosts

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

FUNCTIONALITY OF FUNGI

- Pathogenesis (plants)

- Secondary metabolites



- Symbiosis

- Heterotrophy

FUNCTIONALITY OF FUNGI

- Pathogenesis (humans)

-- 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”

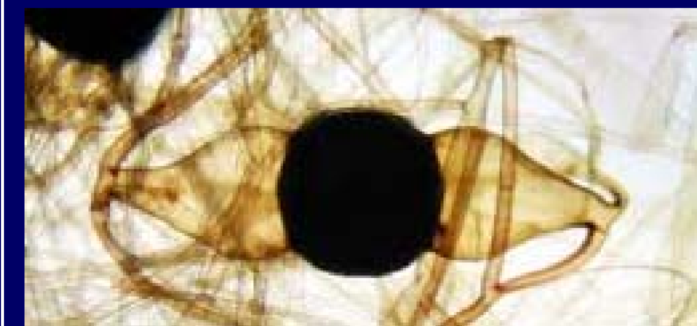
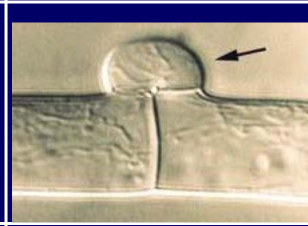
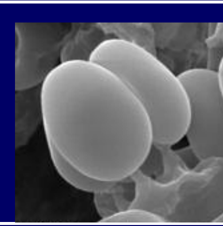
PAST TAXONOMY

- Mode of reproduction important

Holomorph	
Teleomorph	Anamorph
Sexual reproduction	Asexual reproduction
Fusion of two nuclei into zygote	Mitosis
Perfect Fungi	“Fungi Imperfecti”
Filobasidiella neoformans	*Cryptococcus neoformans*

- Type of sexual reproduction important

PAST TAXONOMY

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

CURRENT TAXONOMY

Phylum Zygomycota	Subphylum Mucoromycotina *Rhizopus, Mucor, Lichtheimia* (*Absidia*)
	Subphylum Entomophthoromycotina *Conidiobolus, Basidiobolus*
Phylum Basidiomycota	Phylum Basidiomycota *Cryptococcus, Trichosporon* anamorphs
Phylum Ascomycota	Phylum Ascomycota 80% of medically-important fungi
Phylum Deuteromycota	Not widely utilized due to PCR

CLASSIFICATION OF FUNGI

- Taxonomy
- Disease
  - Deep-seated (disseminated) mycoses
  - Opportunistic mycoses
    - Aspergillosis
    - Candidiasis
    - Mucormycosis
  - Subcutaneous mycoses
    - Mycetoma
    - Chromoblastomycosis
  - Superficial mycoses
    - Dermatomycosis
    - Tinea
    - Onychomycosis
    - Piedra

CLASSIFICATION OF FUNGI

- Taxonomy
- Disease
- Cell morphology (conidiogenesis)

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

CLASSIFICATION OF FUNGI

- Taxonomy
- Disease
- Cell morphology (conidiogenesis)
- Colonial morphology

    Yeast
    Mo(u)ld

UNIFYING CONCEPTS

Microscopic observation from primary specimens

Direct detection from primary specimens

Presumptive identification
(colony, growth distribution, cell morphology)

Rapid diagnostics

Definitive identification

The Pretenders

30-year-old Male with HIV

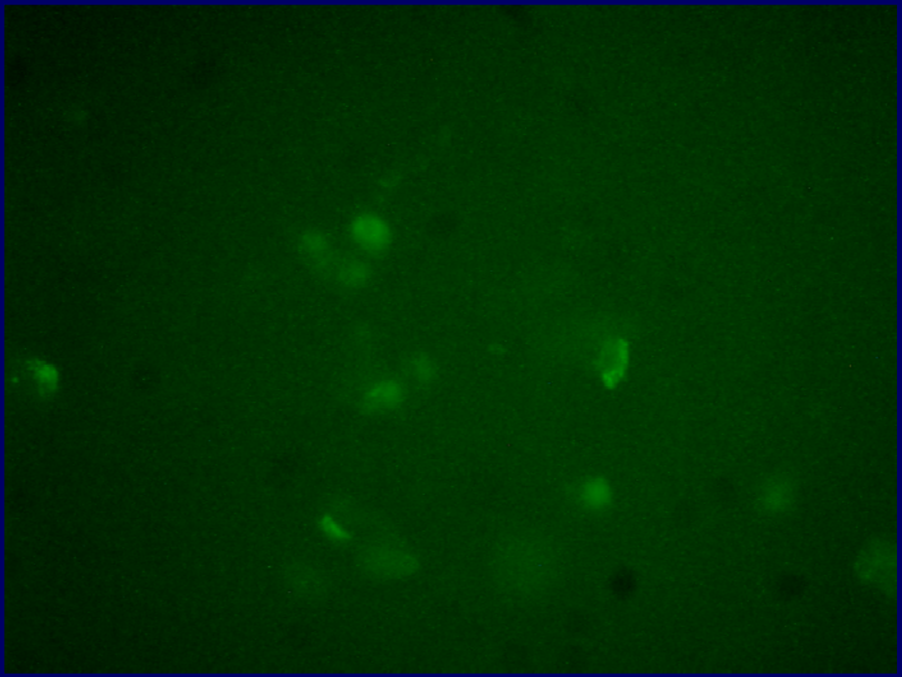
- Shortness of breath, productive cough, night sweats, poor oral intake, weight loss
- Vitals

  T_{max} 101° F
  Tachycardia (95/min)
  Tachypnea (22/min)
  Blood pressure 103/63

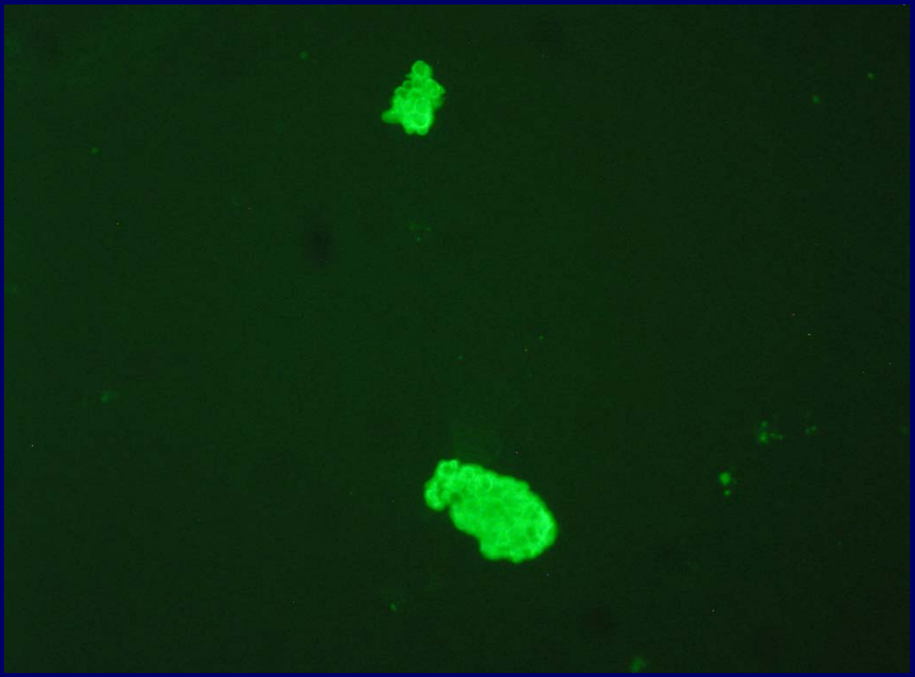
- Studies

  pO_2 87% on room air
  Albumin 2.9 g/dL
  CD4 count 17/mm^3
  Bilateral diffuse infiltrates

J. Clin. Microbiol. **49:** 3113; 2011



Expectorated sputum



Bronchoalveolar lavage

J. Clin. Microbiol. **49:** 3113; 2011

Pneumocystis jirovecii (formerly *carinii*)

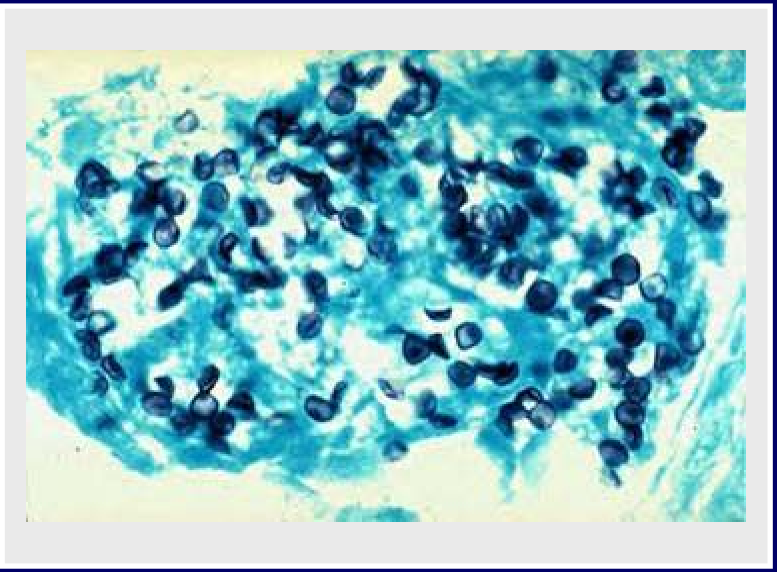
- Protozoan?? — Antifungals ineffective; No ergosterol; "Life cycle"

  Fungus?? — Chitin; ~Ascospores internally; Stains with "fungus" stains

  Bacterium?? — Trimethoprim-sulfamethoxazole
- Different species infect different hosts
- Nothing *in vitro*

Pneumocystis jirovecii (formerly *carinii*)

- Serology, nucleic acid detection not useful
- Cyst wall, intracystic body stains



Gomori methenamine silver

Giemsa

***Pneumocystis jirovecii* (formerly *carinii*)**

- 98.4% agreement between toluidine blue (histology) and fluorescent monoclonal antibody (microbiology)

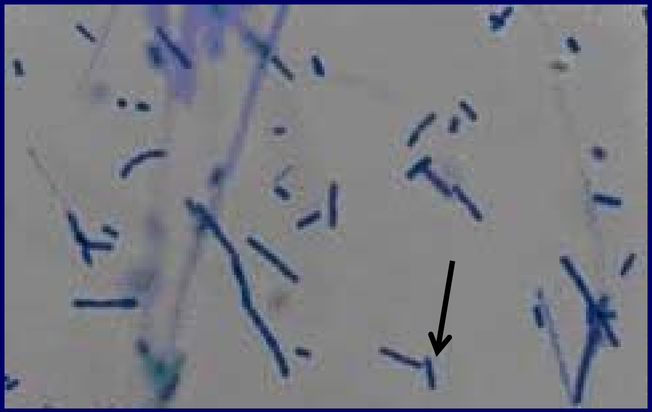
J. Clin. Microbiol. **25:** 1837-1840; 1987

- 15% sensitivity in a cohort of expectorated and induced sputum specimens collected from AIDS population

Arch. Pathol. Lab. Med. **112:** 1229-1232; 1988

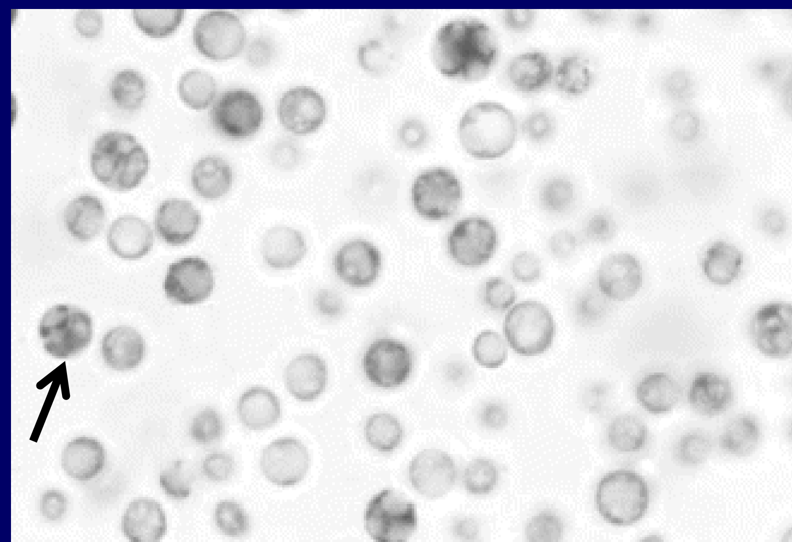
Geotrichum spp.

- Yeast-like mold
- Debilitated hosts; bronchial manifestations, rare disseminated disease
- Inhibited by cycloheximide; urease-negative



Prototheca wickerhamii

- Yeast-like achlorophyllous alga
- Verrucous cutaneous infections
  Olecranon bursitits
- Sporangia with septations



Clin. Infect. Dis. **48:** 1160-1161; 2009

"BLACK YEASTS"

- Able to produce melanized budding cells at some (early) stage in life cycle
- Most also produce true mycelium; therefore, identification based on asexual reproduction
- Many examples within Ascomycota and Basidiomycota; lots of name changes

  Exophiala spp.
  Aureobasidium pullulans
  Hortaea werneckii

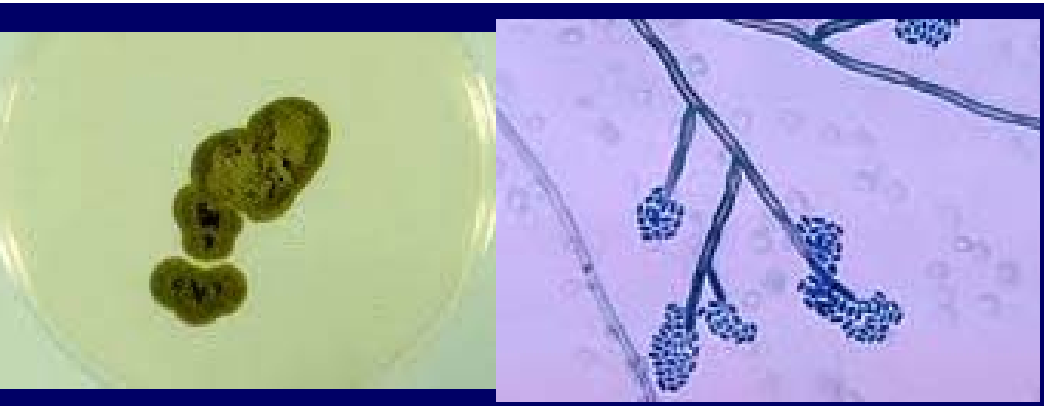
"BLACK YEASTS"

- Slow-growing; some are very slow-growing
- Differential optimum growth temperatures
- *E. jeanselmei* mycetoma
  phaeohyphomycosis

  E. dermatitidis phaeohyphomycosis
  predilection for CNS; ocular

  H. werneckii tinea nigra

  A. pullulans common contaminant
  phaeohyphomycosis



Exophiala jeanselmei

Exophiala dermatitidis

Aureobasidium pullulans

Hortaea werneckii

“BLACK YEASTS”

Organism	Casein utilization	Tyrosine utilization	Growth in 15% NaCl	KNO_3 assimilation	Maximum growth temperature
E. jeanselmei	-	+	-	+	≤ 37° C
E. dermatitidis	-	+	-	-	42° C
H. werneckii	+	-	+	+	Variable

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

Ustilago spp.

- "Corn smut fungus"
- Inhaled and subsequently isolated from sputum; rarely implicated in human disease
- White, pasty, moist, yeast-like at first; becomes tan/brown, mycelial within 20 days

The Contenders

Rhodotorula spp.

- Proclivity to impact terminal stages of carcinoma and bacterial endocarditis
- Urease-positive; rare rudimentary pseudohyphae
- *R. mucilaginosa* utilizes KNO_3; *R. glutinis* -

Sporobolomyces spp.

- Rare pathogenesis in immunocompromised patients
- Best growth at 25° C; forcible discharge of kidney-shaped ballistoconidia, forms satellite colonies

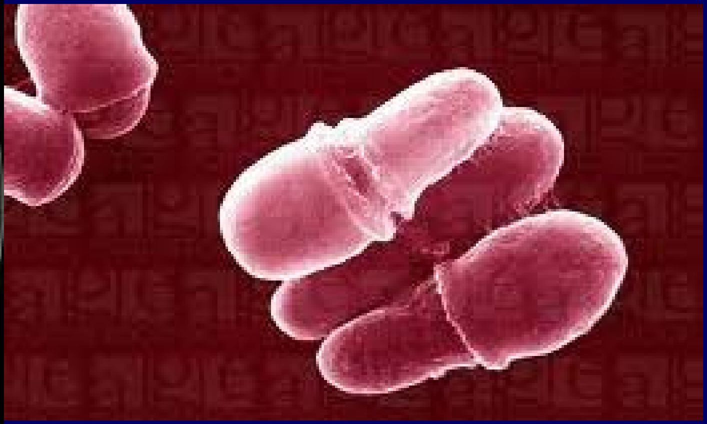
Malassezia furfur

- Normal skin flora in more than 90% of adults
- Tinea versicolor; cradle cap; dandruff

  Catheter-related sepsis (neonates, TPN) with secondary pneumonia
- Optimal recovery of the organism involves acquisition of blood via lipid infusion catheter
- Grows poorly at 25° C; solid medium overlaid with thin layer of olive oil (not for veterinary)

Malassezia furfur

Tinea versicolor



Cells round at one end and bluntly cut at other (no constriction);

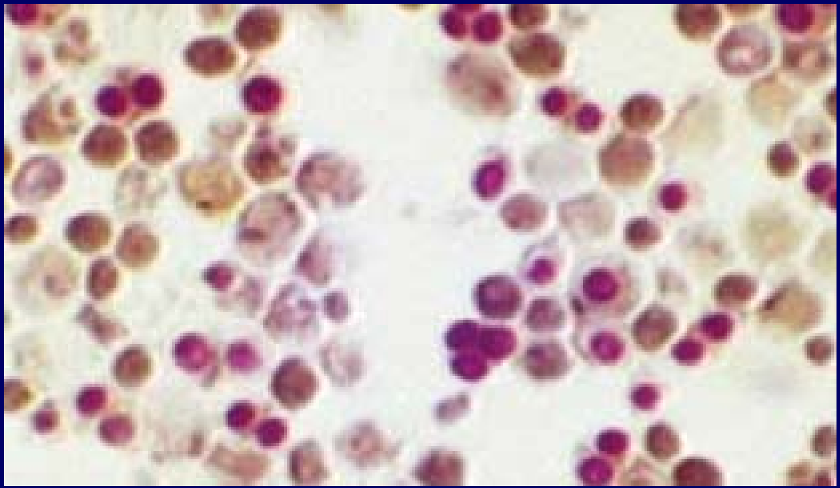
Collarette difficult to discern by conventional microscopy

Urease-positive

Saccharomyces cerevisiae

- Typically non-pathogenic
- Exposure to commercial strains associated with health foods and baking may allow for colonization/infection

J. Clin. Microbiol. **36:** 557-562; 1998



1-4 ascospores per ascus

Stain Gram-negative (vegetative cells stain Gram-positive)

Stain with Kinyoun stain (vegetative cells visualized with counterstain)

Best demonstrated on specialized medium

Trichosporon spp.

- *T. beigelii* formerly considered main pathogen of genus
- Major taxonomic revision in 1992
- 19 taxa recognized; nearly all systemic infection caused by six species

T. asahii	*T. asteroides*	*T. mucoides*
T. cutaneum	*T. inkin*	*T. ovoides*

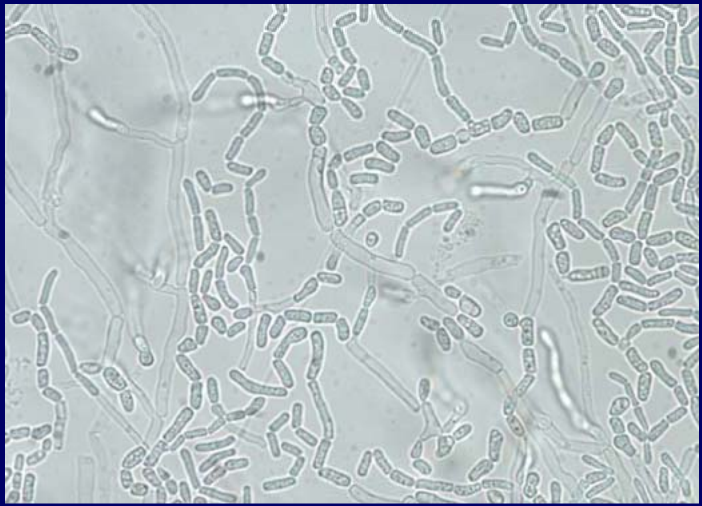
- Neutropenic patients; AIDS; extensive burns; heart valve surgery; catheterized patients

Trichosporon spp.

T. asahii
nutritive medium

T. asahii
chromogenic medium

“Patriot Blue”



T. asahii
cell morphology

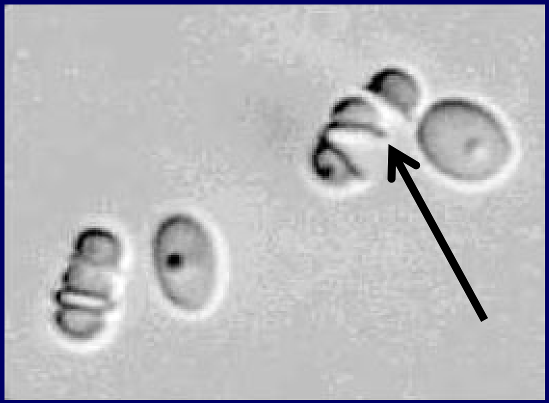
Urease-positive

Blastoschizomyces capitatus

- Obsolete taxa *Trichosporon capitatum* and *Blastoschizomyces pseudotrichosporon*
- Emerging cause of invasive fungal disease in leukemic patients; mortality rate from invasive disease high in neutropenic patients
- Difficult to delineate from *Trichosporon*

Urease-negative	Growth at 45° C
Non-fermentative	Growth on cycloheximide

“Current” Designation	“Former” Designation
Clavispora lusitaniae teleomorph	*Candida lusitaniae* anamorph
Yarrowia lipolytica teleomorph	*Candida lipolytica* anamorph
Issatchenkia orientalis teleomorph	*Candida krusei* anamorph
Candida kefyr	*Candida pseudotropicalis*
Debaryomyces hansenii teleomorph	*Candida famata* anamorph
Pichia guilliermondii teleomorph	*Candida guilliermondii* anamorph
Hansenula anomala (now obsolete)	*Candida pelliculosa*
Pichia anomala teleomorph	*Hansenula anomala* (now obsolete)
Wickerhamomyces anomalus	*Pichia anomala*



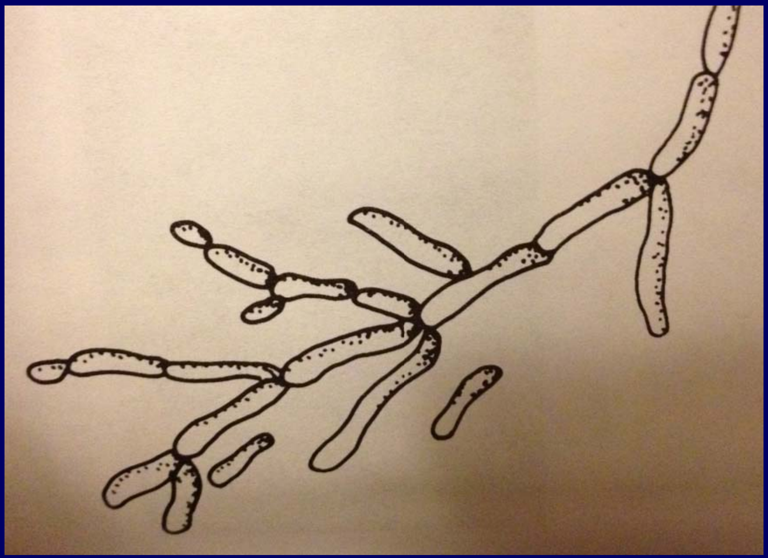
Wickerhamomyces species

1-4 ascospores per ascus

Brim that turns downward around each ascospore (helmet)

Candida lipolytica

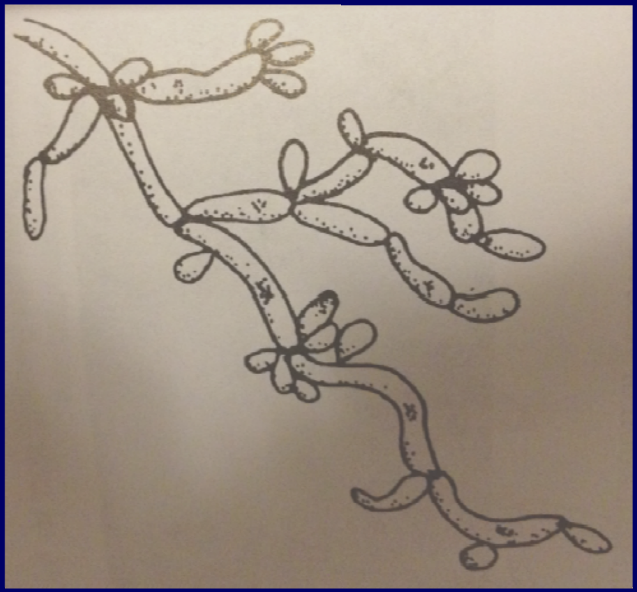
- Emerging opportunistic pathogen
- Pseudohyphae and true hyphae bearing elongate blastoconidia form stark branching appearance; urease-positive



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

Candida lusitaniae

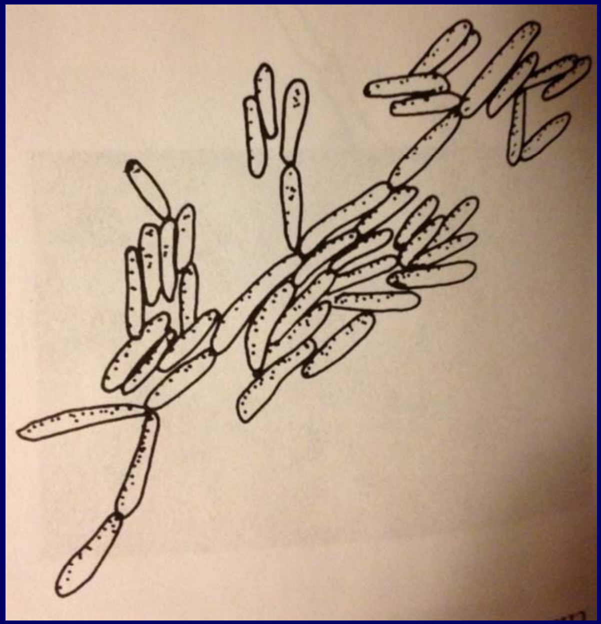
- Emerging opportunist (malignancy); highly-resistant to amphotericin B
- Pseudohyphae slender, branched, curved; short chains of elongate blastoconidia



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

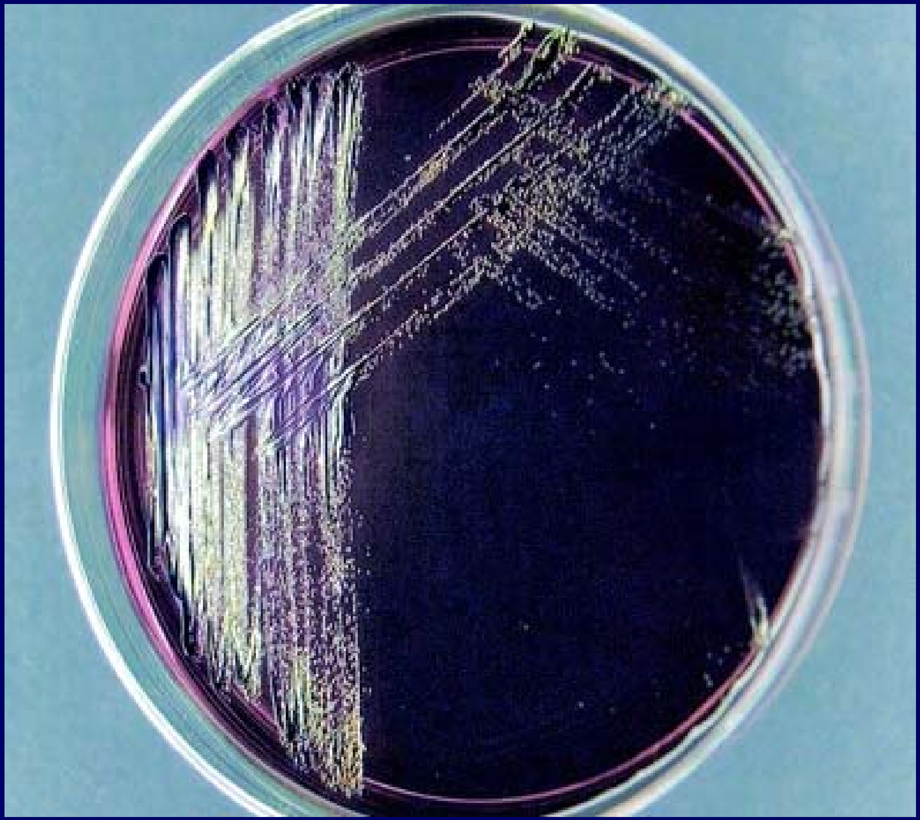
Candida kefyr

- Rare etiology of systemic disease, cystitis
- Elongate blastoconidia line up in parallel; "logs in stream"



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

Candida kefyr

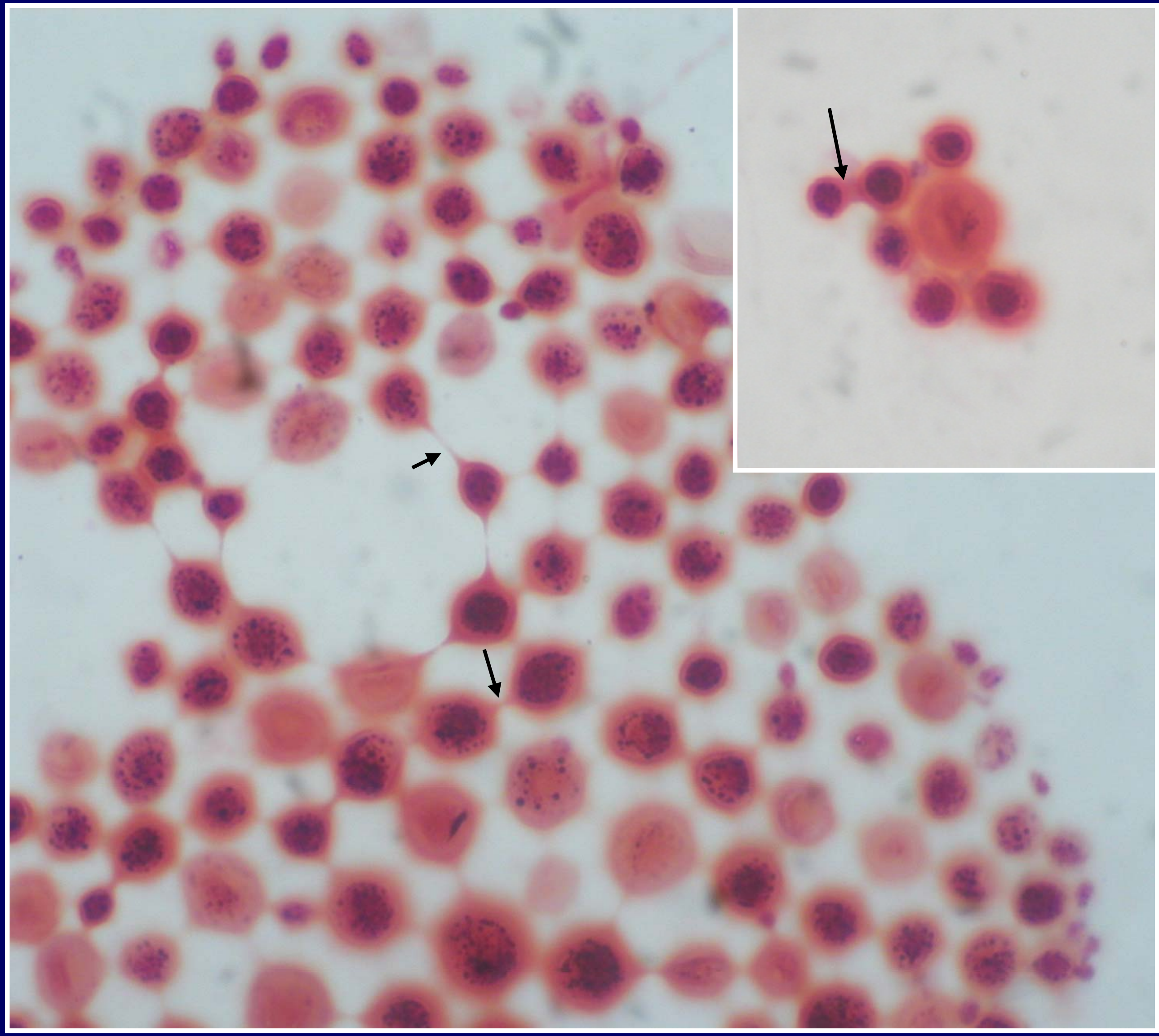


Presence of metallic green sheen on Levine eosin methylene blue (EMB) agar demonstrated 100% positive predictive value for *C. kefyr*

J. Clin. Microbiol. **40:** 4281-4284; 2002

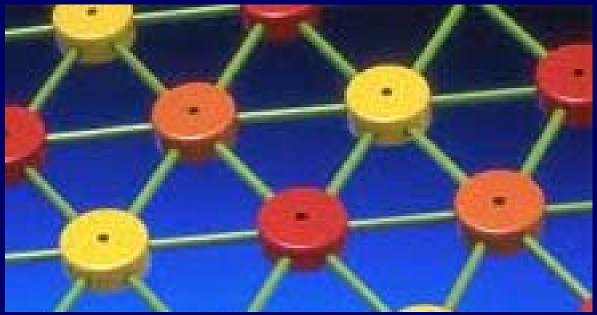
38-year-old Male with GI & Headache

- 3-month history of GI distress, headache; fever, chills, poor appetite, 20-pound weight loss over three months
- Past contact with commercial sex worker
- CT imaging of abdomen consistent with fecal impaction; MRI had increased signal intensity in periventricular and white matter
- CSF protein 84 mg/dL (12-60) glucose 4 (40-70)
  22 leukocytes (0-5); 94% lymphocytes



38-year-old Male with GI & Headache

- Initially reported as “Abundant yeast”
- Tinkertoy® “appreciation” amended report



- Definitive identification as *Cryptococcus neoformans*
- Cryptococcal antigen titer (CSF) 1024
- Patient also had CD4 count 29/mm^3, HIV load ~10^5 copies, thrush, Kaposi sarcoma

Cryptococcus neoformans

- HIV signal disease; cryptococcosis is first AIDS-defining illness in 45% of AIDS patients
- CNS disease, skin, bone, lung, other organs
- CSF cryptococcal antigen sensitivity equals or exceeds that of culture

S. Afr. Med. J. **71:** 510-512; 1987
J. Clin. Microbiol. **32:** 1680-1684; 1994

- 85.2% sensitivity of Gram stain in culture-positive cases of cryptococcal meningitis

J. Clin. Microbiol. **36:** 1617-1620; 1998

Cryptococcus spp.

- *C. neoformans*/*gattii* complex reclassification (e.g., *C. neoformans* var. *neoformans*)
- Six species may be encountered clinically

C. neoformans	*C. albidus*	*C. terreus*
C. uniguttulatus	*C. luteolus*	*C. laurentii*

- Only *C. neoformans*/*gattii* complex produces phenol oxidase; "birdseed" agar, also a rapid test utilizing caffeic acid disc
- Urease-positive; nitrate can help differentiate

NORTH AMERICA CANDIDEMIA

Species	Percentage Distribution		
	2008-2009	1997	1952-1992†
C. albicans	43.4	55.3	54.0
C. glabrata	23.5	17.0	8.0
C. parapsilosis	17.1	12.1	7.0
C. tropicalis	10.5	7.2	25.0
C. krusei	1.6	2.3	4.0

† Estimate

J. Clin. Microbiol. **36:** 1886-1889; 1998
J. Clin. Microbiol. **49:** 396-399; 2011

CANDIDEMIA BY AGE

Species	Percentage Distribution	
	0-19 year olds	80-99 year olds
C. albicans	50.0	46.7
C. glabrata	2.0	28.6
C. parapsilosis	28.5	17.1
C. tropicalis	12.9	3.8
C. krusei	0.8	2.9

Diagn. Microbiol. Infect. Dis. **68:** 278-283; 2010

NOSOCOMIAL CANDIDEMIA

Species	Percentage Distribution	
	ICU	Non-ICU
C. albicans	50.4	47.4
C. glabrata	17.5	18.1
C. parapsilosis	15.1	18.9
C. tropicalis	10.5	9.6
C. krusei	2.1	2.1

Int. J. Antimicrob. Agents **38:** 65-69; 2011

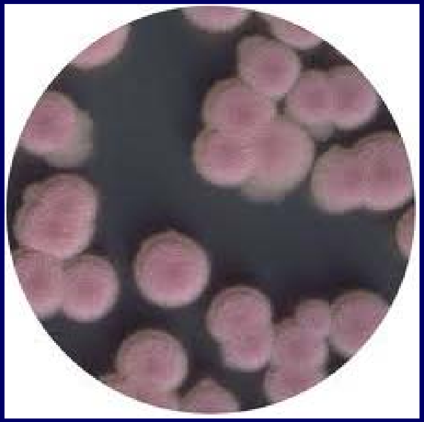
EPIDEMIOLOGY OF CANDIDEMIA

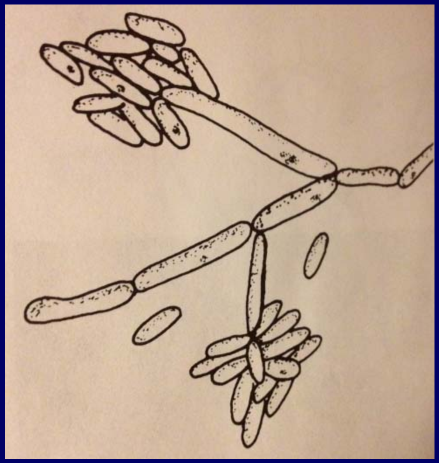
Species	Percentage Distribution	
	Nosocomial Onset	Community Onset
C. albicans	47	51
C. glabrata	18	18
C. parapsilosis	18	15
C. tropicalis	11	11
C. krusei	3	1

Antimicrob. Agents Chemother. **55:** 561-566; 2011

Candida krusei

- Emerging opportunist; constitutively resistant to fluconazole
- Distinctive morphology on chromogenic medium
- Urease-positive (some)
- Elongate blastoconidia, tree-like appearance

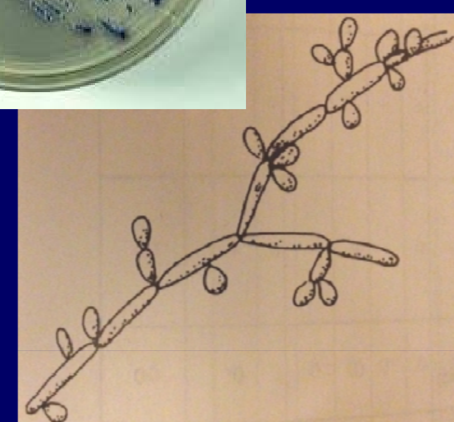




D. H. Larone, Medically Important Fungi, fourth ed.

Candida tropicalis

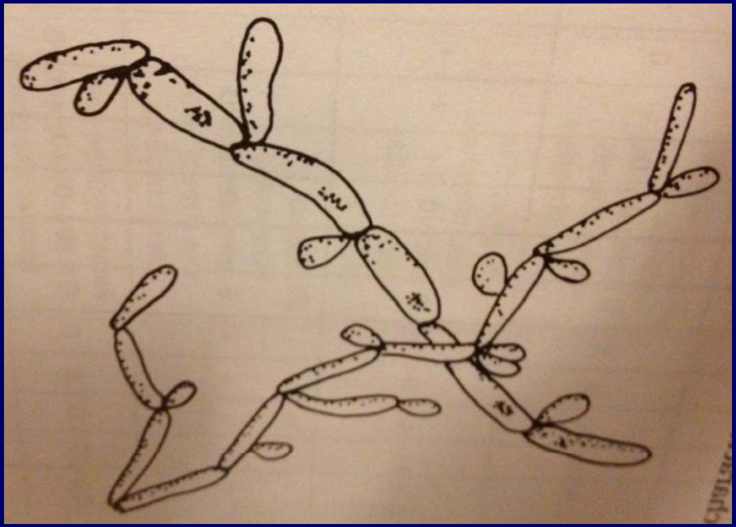
- In patients with lymphoreticular malignancy or leukemia, more virulent than *C. albicans*
- Distinctive morphology on chromogenic medium
- Blastoconidia singly or in small groups along long pseudohyphae



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

Candida parapsilosis

- Infections in particulary susceptible hosts; candidal endocarditis
- Blastoconidia singly or in small clusters; crooked/curved short pseudohyphae



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

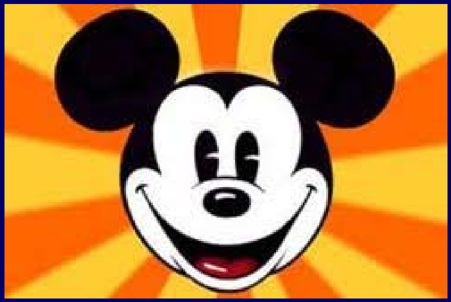
Candida glabrata

- Potential induction of fluconazole resistance upon suboptimal treatment
- Endocarditis, meningitis, multifocal disease
- 20% of *Candida* urinary tract infections
- When compared to CFU on blood agar, *C. glabrata* CFU on eosin methylene blue agar are larger

Diagn. Microbiol. Infect. Dis. **28:** 65-67; 1997

Candida glabrata

- Small blastoconidia; may bud at 11:00 and 1:00

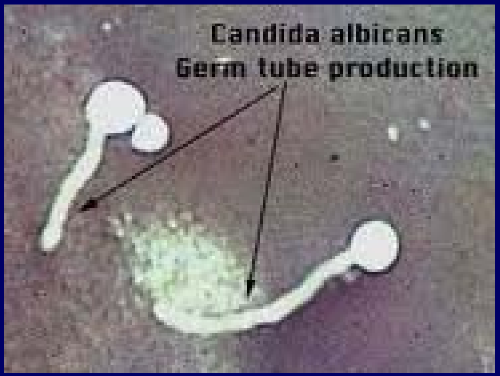


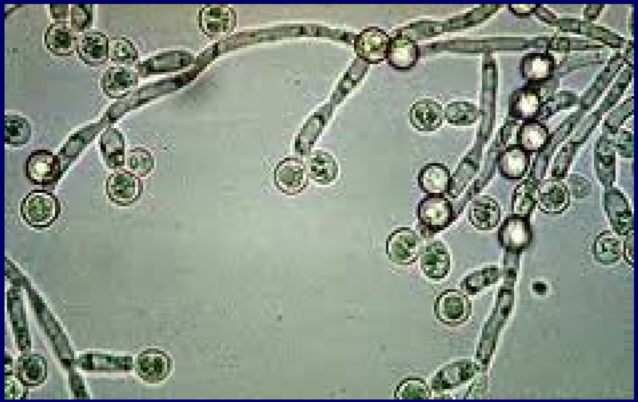
- "Distinctive" morphology on chromogenic medium

- Rapid trehalose testing gives presumptive ID within 3 hours when correlated with cell morphology; watch out for blood agar

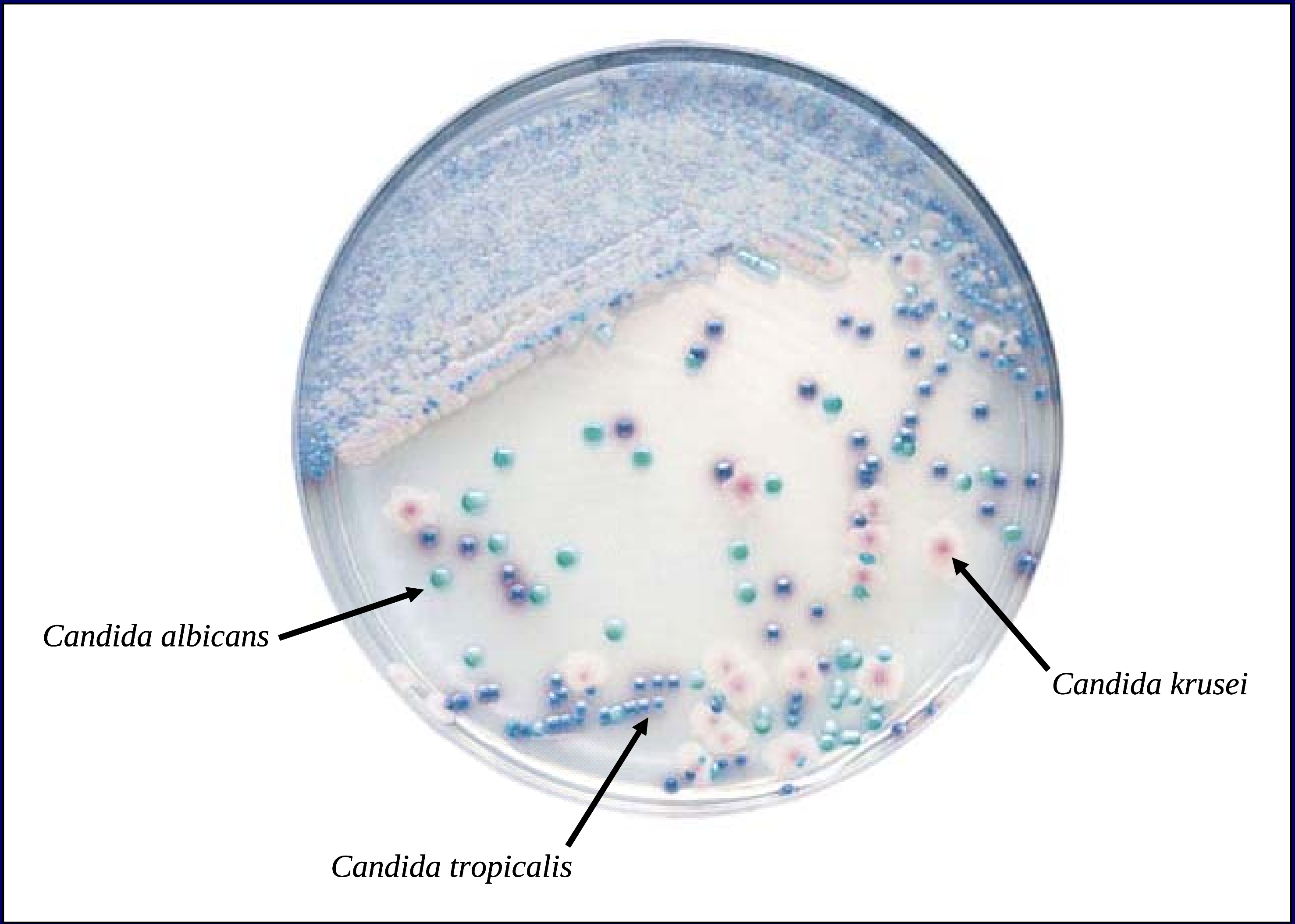
Candida albicans

- Most common species isolated from all forms of candidiasis
- Make sure that germ tube contiguous (*C. tropicalis*); considered presumptive
- Distinctive morphology on chromogenic medium
- Terminal chlamydoconidia, especially at 25° C





CHROMOGENIC MEDIUM



J. Clin. Microbiol. **32:** 1923-1929; 1994
J. Clin. Microbiol. **34:** 56-61; 1996

Candida dubliniensis

- Most frequently isolated from oropharynx of HIV-positive patients (pseudomembranous oral candidiasis)
- Infrequently recovered from blood, urine, vaginal specimens (immunocompromised)
- *Candida albicans* mimicry

  germ tubechlamydoconidia
- Commercial germ tube reagent reduces frequency of GT-positive *C. dubliniensis*

J. Clin. Microbiol. **43:** 2465-2466; 2005

TO ID OR NOT TO ID??

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2011, p. 4415 Vol. 49,
0095-1137/11/$12.00 doi:10.1128/JCM.05923-11

Do Hospital Microbiology Laboratories Still Need To Distinguish *Candida albicans* from *Candida dubliniensis*?

- *C. dubliniensis* first described in 1995, midst AIDS epidemic and introduction of HAART
- As a result, mucocutaneous candidiasis commonly seen; many of first strains resistant to fluconazole (or inducible)

TO ID OR NOT TO ID??

- Original *C. dubliniensis* discoverers note that yeast is fairly susceptible to fluconazole

Biochem. Soc. Trans. **33:** 1210-1214; 2005

- Data corroborated by:

CDC	4.8% resistance in 42 isolates
Denmark	3.1% resistance in 65 isolates
Global surveillance	3.9% resistance in 310 isolates

J. Clin. Microbiol. **49:** 4415; 2011
J. Clin. Microbiol. **49:** 325-334; 2011
J. Clin. Microbiol. **48:** 1366-1377; 2010

DEFINITIVE IDENTIFICATION

- Carbohydrate assimilation is mainstay; Wickerham and Burton method has given way to commercial kits (some automated)

Candida spp.
Cryptococcus spp.
Saccharomyces cerevisiae
Rhodotorula spp.
Trichosporon spp.
(former) *Pichia* spp.
Geotrichum spp.
Malassezia furfur
Sporobolomyces spp.
Prototheca wickerhamii

- PNA FISH from positive blood cultures (*C. albicans*, *C. glabrata*, *C. tropicalis*); does not replace subculture (polymicrobial)

MALDI-TOF

- Spectra of 247/267 clinical isolates correlated with API biochemical profiling; Big Five did very well (n = 220)

J. Clin. Microbiol. **48:** 2912-2917; 2009

- Prospective study of 61 yeast isolates yielded 96.8% correct ID to genus (*P* = 0.03 versus biochemical methods); 84.0% correct ID to species

J. Clin. Microbiol. **48:** 900-907; 2010

Antifungal Susceptibility Testing

CLSI DOCUMENTS OF INTEREST

- M27-A3 Reference Method for Broth Dilution Susceptibility Testing of Yeasts, 3rd ed. Approved Standard

- M44-A2 Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, 2nd ed. Approved Guideline

BROTH MICRODILUTION

- Can test variety of yeasts, including *Candida* and *C. neoformans*, but not dimorphs
- Interpretive criteria only for *Candida* spp.

Agent	**Minimum Inhibitory Concentration (μg/mL)**				
	Susceptible	Susceptible (dose-dependent)	Intermediate	Resistant	Non-susceptible
Fluconazole	≤ 8	16-32		≥ 64	
Voriconazole	≤ 1	2		≥ 4	
Itraconazole	≤ 0.125	0.25-0.50		≥ 1	
5-fluorocytosine	≤ 4		8-16	≥ 32	
Anidulafungin	≤ 2				> 2
Caspofungin	≤ 2				> 2
Micafungin	≤ 2				> 2

CLSI M27-A3

BROTH MICRODILUTION

- RPMI 1640 broth (MOPS buffer, 0.2% dextrose)
- 24h *Candida* growth; 48h *C. neoformans*
  Sabouraud dextrose, potato dextrose agar
- 0.5 McFarland; dilution to final inoculum range of 0.5 x 10^3 to 2.5 x 10^3 CFU/mL
- Fluconazole, 5-fluorocytosine 0.12-64 μg/mL
  Other antifungals 0.03-16 μg/mL

CLSI M27-A3

BROTH MICRODILUTION

- 35° C ambient air

  24 hours for echinocandins
  24-48 hours for amphotericin B
  24-48 hours for fluconazole
  48 hours for 5-fluorocytosine
  48 hours for other azoles
  70-74 hours for *C. neoformans* testing

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

CLSI M27-A3

DISK DIFFUSION

- Has lagged behind broth dilution
- *Candida* versus caspofungin posaconazole fluconazole voriconazole

Agent	Zone Diameter (mm)			
	Susceptible	Susceptible (dose-dependent)	Resistant	Non-susceptible
Caspofungin	≥ 11			≤ 10
Fluconazole	≥ 19	15-18	≤ 14	
Voriconazole	≥ 17	14-16	≤ 13	

CLSI M44-A2

DISK DIFFUSION

- Mueller-Hinton agar with 2% dextrose (0.5 μg methylene blue/mL)
- 24-hour *Candida* spp. growth on Sabauroud Dextrose agar
- 0.5 McFarland standard for inoculum of 1×10^6 to 5×10^6 CFU/mL
- Caspofungin 5 μg
  Fluconazole 25 μg
  Posaconazole 5 μg
  Voriconazole 1 μg

CLSI M44-A2

DISK DIFFUSION

- 35° C ambient air; 20-24 hours
- Observe for prominent reduction in growth
  - Ignore pinpoint microcolonies at zone edge
  - Ignore large colonies within inhibition zone

CLSI M44-A2

Etest

- Fluconazole, itraconazole, 5-fluorocytosine strips FDA-approved for clinical use
- Etest MIC data tended to be higher than broth microdilution for fluconazole testing of 1586 *Candida* spp., but overall agreement 96.4%
- Etest MIC data tended to be higher than broth microdilution for voriconazole testing of 1586 *Candida* spp., but overall agreement 98.1%

J. Clin. Microbiol. **41:** 1440-1446; 2003

FLUCONAZOLE RESISTANCE

Species	Percentage Resistant	
	1997	2008-2009
C. albicans	0.6	0.1
C. glabrata	8.7	5.6
C. parapsilosis	0.0	5.0
C. tropicalis	0.0	3.2
C. krusei	100.0	100.0

J. Clin. Microbiol. **36:** 1886-1889; 1998
J. Clin. Microbiol. **49:** 396-399; 2011

FLUCONAZOLE RESISTANCE

Species	Percentage Resistant	
	Nosocomial Onset	Community Onset
C. albicans	0.0	0.0
C. glabrata	7.7	3.3
C. parapsilosis	5.8	6.6
C. tropicalis	3.3	0.0
C. krusei	100.0	100.0

Antimicrob. Agents Chemother. **55:** 561-566; 2011

FLUCONAZOLE RESISTANCE

Species	Percentage Resistant	
	ICU	Non-ICU
C. albicans	0.0	0.0
C. glabrata	5.9	6.3
C. parapsilosis	6.8	4.3
C. tropicalis	4.9	2.2
C. krusei	100.0	100.0

Int. J. Antimicrob. Agents **38:** 65-69; 2011

THE END

- Lots of name changes
- Several aids in presumptive identification of yeast; baseline observations can also lend validity/correlation to an automated result
- Antifungal susceptibility testing for yeast continues to be a work in progress

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