Fungal Infections : Still Neglected

NACMID Annual Conference

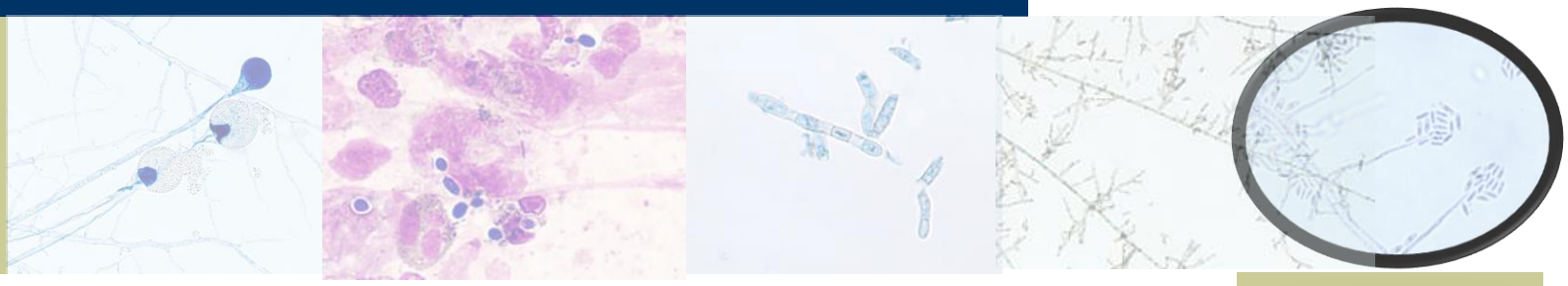
September 26, 2023

Lowell, Massachusetts

Laboratory Profession

Northeast Association for Clinical Microbiology and Infectious Diseases - NACMID - Founded 1953

UMASS



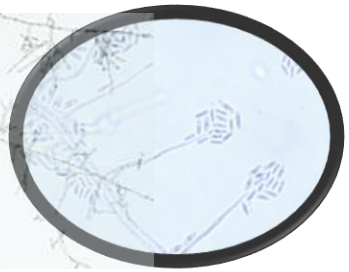
- James T. Griffith Ph.D., CLS (NCA)
  - Chancellor Professor Emeritus
- University of Massachusetts
  - Managing Partner
- Forensic DNA Associates, LLC

Laboratory Profession

FDA

Forensic DNA Associates

Learning Objectives



- 1. Describe the current global concerns about fungal infections
- 2. Discuss the current and future importance of scaled-up clinical laboratory identification of fungi
- 3. Describe how invasive fungal diseases are associated with immunocompromised patient conditions

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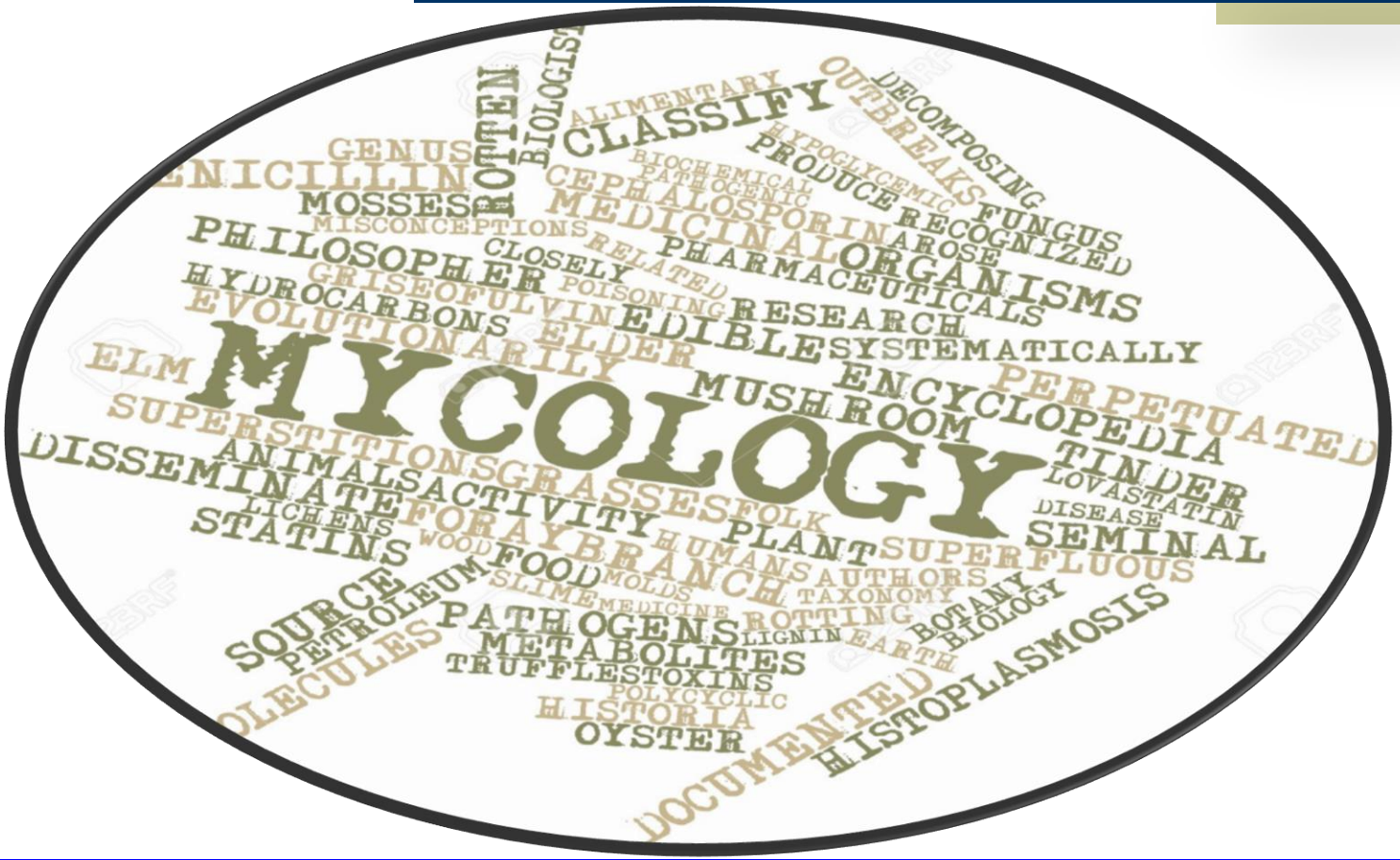
Scope

Scope


MYCOLOGY
CLASSIFY
ROTTEN
BIOLOGIST
ALIMENTARY
OUTBREAKS
DECOMPOSING
FUNGUS
RECOGNIZED
PRODUCE
ROSE
GENUS
PENICILLIN
BIOCHEMICAL
PATHOGENIC
CEPHALOSPORIN
MOSSES
MEDICINAL
ORGANISMS
MISCONCEPTIONS
RELATED
PHILOSOPHER
CLOSELY
PHARMACEUTICALS
GRISEOFULVIN
POISONING
HYDROCARBONS
RESEARCH
EVOLUTIONARILY
ELDER
EDIBLE
SYSTEMATICALLY
ELM
MUSHROOM
ENCYCLOPEDIA
PERPETUATED
SUPERSTITIONS
GRASSES
FOLK
TINDER
LOVASTATIN
DISSEMINATE
ANIMALS
ACTIVITY
DISEASE
LICHENS
FORAY
BRANCH
PLANT
SUPERFLUOUS
SEMINAL
STATINS
WOOD
HUMANS
AUTHORS
FOOD
MOLDS
TAXONOMY
SLIME
MEDICINE
ROTTING
BOTANY
SOURCE
PETROLEUM
PATHOGENS
LIGNIN
EARTH
BIOLOGY
METABOLITES
TRUFFLES
TOXINS
POLYCYCLIC
HISTORIA
OYSTER
DOCUMENTED
HISTOPLASMOSIS

Scope

- Aren't we supposed to be "protected" from fungi?
  - Our mammalian core temperature is too "hot" for them
  - **Comparatively cool skin temp, perhaps some fungi can get with;**
    - Athlete's Foot
    - Yeast
    - Ringworm
  - Invasive infections should be RARE
- **We forgot that fungi can exploit damaged immune systems, now those people live longer**
  - We actually have "**immunosuppressive**" medical treatments
  - HIV (40 years ago) was actually alerted to all of us because of a co-infection with ***Pneumocystis carinii*** (Now ***Pneumocystis jirovecci***)
- Land-clearing, fungicides in crops (↑ Fungal Resistance)
- Mono-culturing
  - ***Phytophthora infestans***, destroyed the Irish potato crop
    - Zoospores dropped in soil
- Climate change

Scope

- The impact, or burden, of these diseases is ***difficult to estimate*** because:
  - Many fungal diseases go **undiagnosed**
  - There is **no national public health surveillance for common fungal infections**, such as ringworm and vaginal candidiasis
  - There is no national public health surveillance for certain serious fungal infections, such as Aspergillosis and Cryptococcosis
- Best guess ~ 6 Million species

- Note: Many of these numbers likely *underestimate* costs, illnesses, and deaths by a large amount, because fungal diseases ***may not be diagnosed***, they ***may not show up*** in the key data used to prepare these estimates. Also, these numbers *do not capture the substantial impact that fungal infections can have on quality of life*

- Ways to measure the ***burden of fungal diseases*** include:
- Cost
  - **Direct medical costs** are estimated at $6.7 to $7.5 billion yearly (U.S.)
  - **Indirect costs** from premature deaths and missed work or school are estimated at $4 billion
  - **Total costs** are conservatively estimated at $11.5 billion and could be as high as $48 billion
- Number of **healthcare visits**
  - More than 75,000 **hospitalizations** and nearly 9 million **outpatient visits** occur every year for fungal diseases (U.S. Hospital admissions for fungal infections ↑ 8.5%/yr {2019-2021})
- Number of **infections**
  - About 23,000 cases of invasive candidiasis occurred in 2017 (CDC)
  - More than 100,000 cases of coccidioidomycosis occurred in 2014
- Number of **deaths** (CDC)
  - An estimated 7,199 deaths from fungal diseases occurred in 2021

Source: Tanne,J.H.; ***Fungal Infections are Especially Dangerous for COVID-19 Patients, CDC Study Warns***, Br. Med. J.; 381:1378, June 15, 2023

Scope

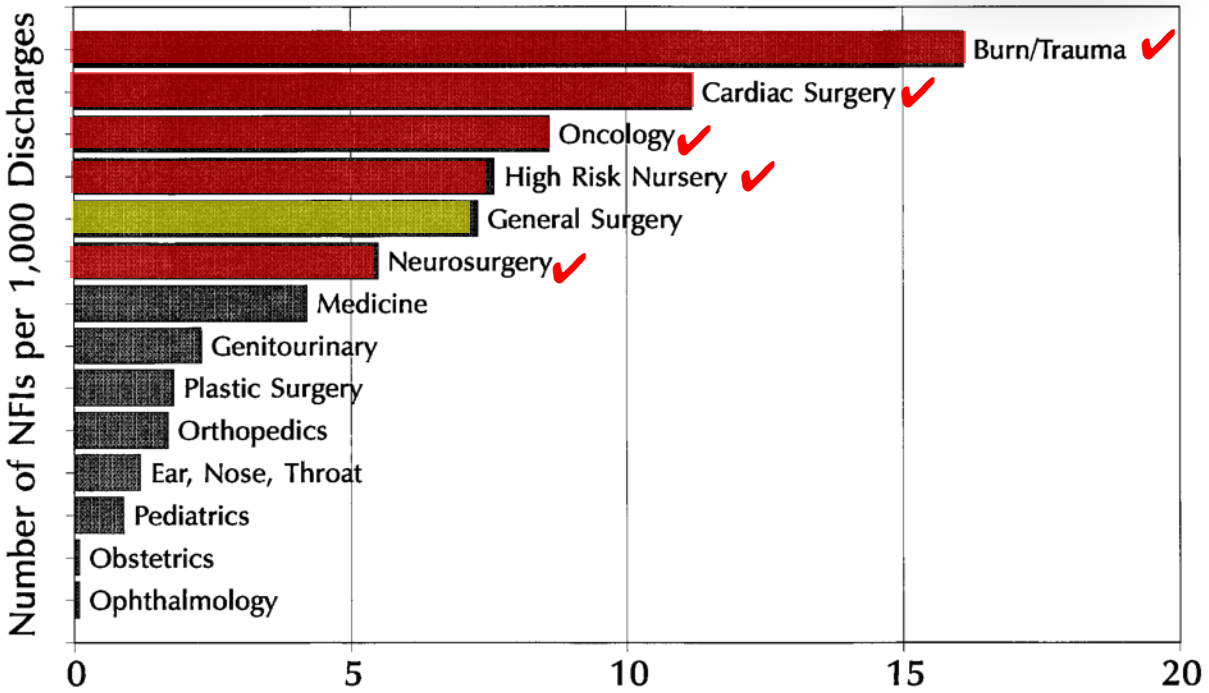
- ~ 6 Million fungal species
- ~120,000 species have been identified
- 250-311 species are known human pathogens
- ~ 300 million people infected (WHO)
- ~ 1.6 million deaths / year (WHO)
  - (more than malaria)
  - (as many as tuberculosis)
- U.S., the CDC estimates;
  - ~ 75,000 people are hospitalized
  - ~ 8.9 million people seek an outpatient visit
  - Costing about $7.2 billion a year

CDC Mycotic Diseases Branch

Scope

- **N**osocomial **F**ungal **I**nfection rate at U.S. hospitals, as reported to the NNIS* system, by the type of hospital ward, 1980 to 1990
- ✔ = likely *immunocompromised patient* via their circumstance

* National Nosocomial Infections Surveillance System

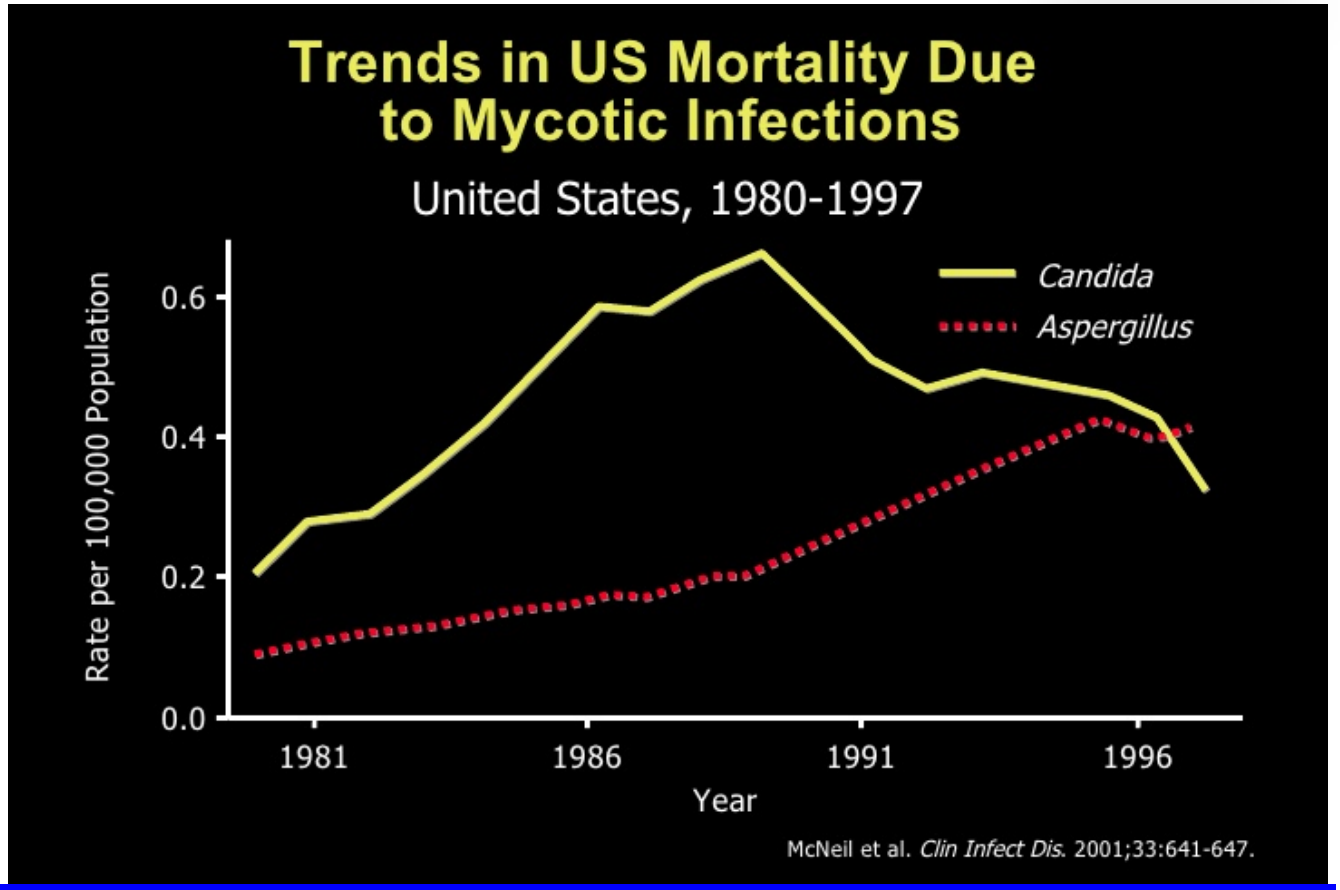


Lest you think during the routine of your day that Microbiology is not so important, bear in mind that world-wide, **21,573 humans die /day** of **infections** (14% of deaths from ALL causes)

Source: Emori,T.G., Culver,D.H., Horan,T.C., et.al.; ***National nosocomial infections surveillance system (NNIS): description of surveillance methods***; Am J Infect Control. 1991 Feb;19(1):19-35

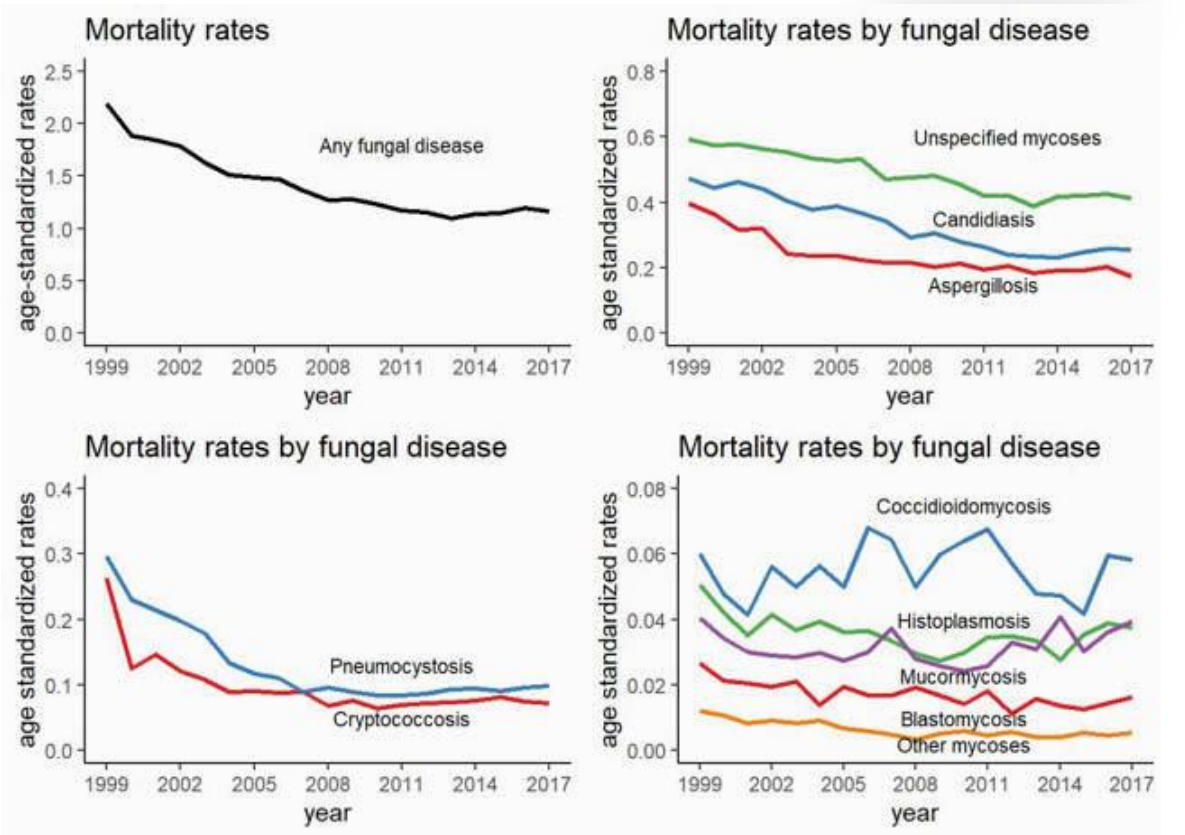
Scope

- 1980-1997



Scope

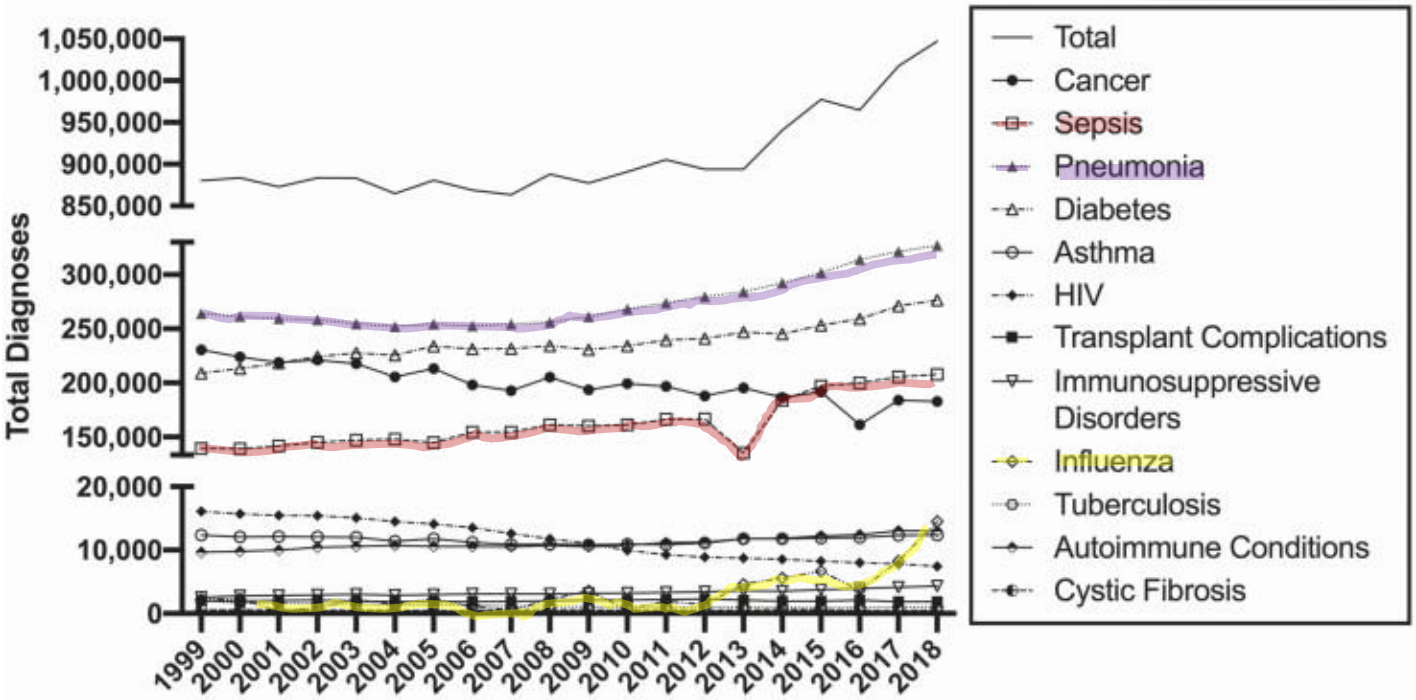
- Since 1999
- Pre-COVID



Source: Mitsuru T. , Jackson, B.R., Deng, L, et.al; Fungal Disease Mortality Trends, United States, 1999–2017; PMC, Pub Med Central, 2020 Oct; 7(Suppl 1): S204, 2020 Dec 31. doi: 10.1093/ofid/ofaa439.458

Immunosuppressive Challenges

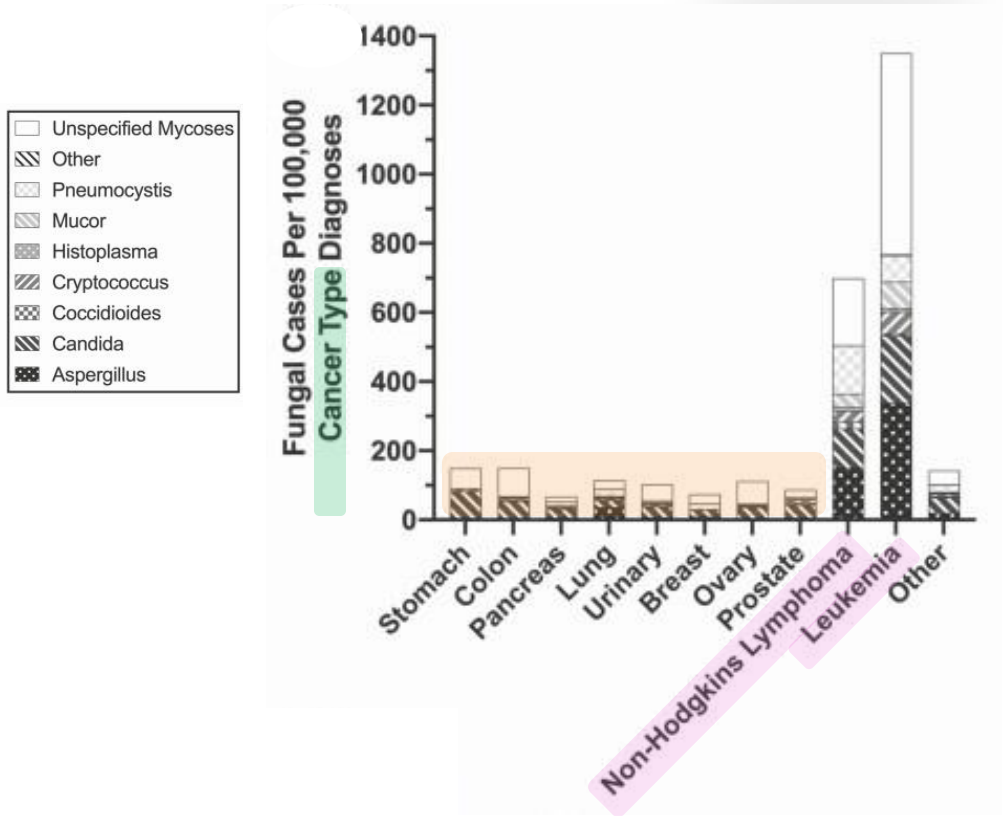
- Since 1999
- Pre-COVID



Source: Rayens,E., Norris,K.A., and Cordero,J.F.; ***Mortality Trends in Risk Conditions and Invasive Mycotic Disease in the United States, 1999–2018***; PMC, Pub Med Central, 2022 Jan 15; 74(2): 309–318

Where are the Fungal Diseases ?

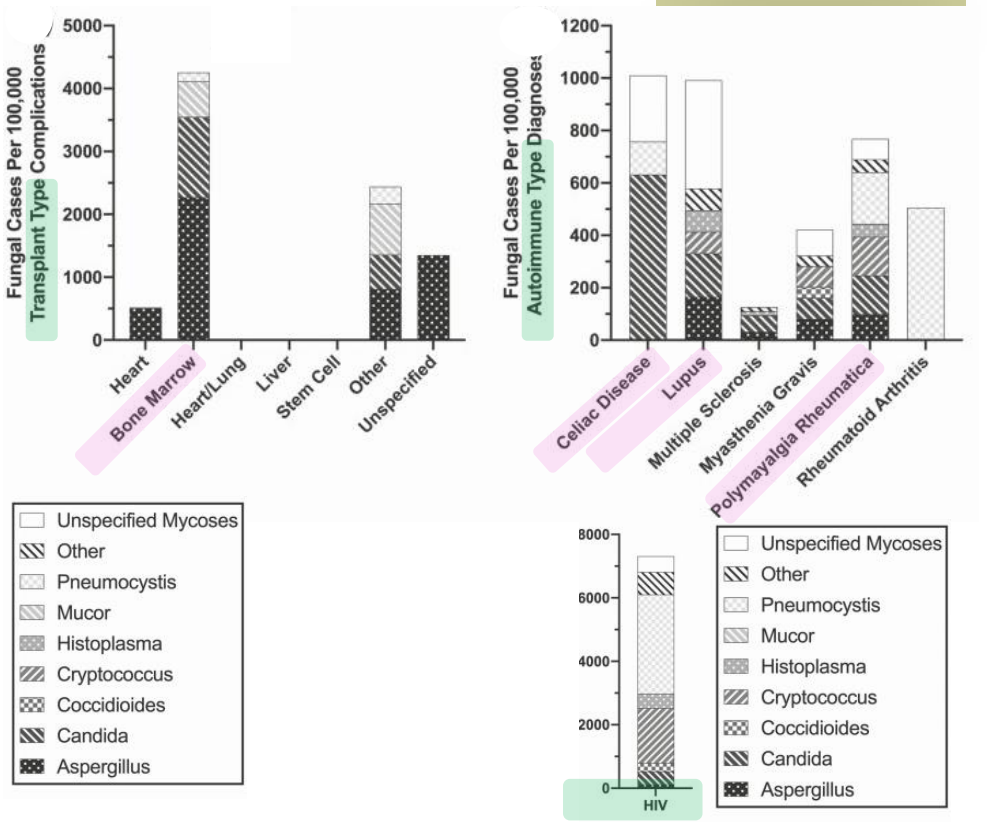
- The burden of fungal infections can be pretty low
- Or unexpectedly high
- This is the new "nich" for fungal infections



Source: Sanguinetti,M., Posteraro,B., Beigelman-Aubry,C., et.al.; **Diagnosis and treatment of invasive fungal infections: looking ahead**
J Antimicrob Chemother. 2019 Mar 1;74(Suppl 2):ii27-ii37

Where are the Fungal Diseases ?

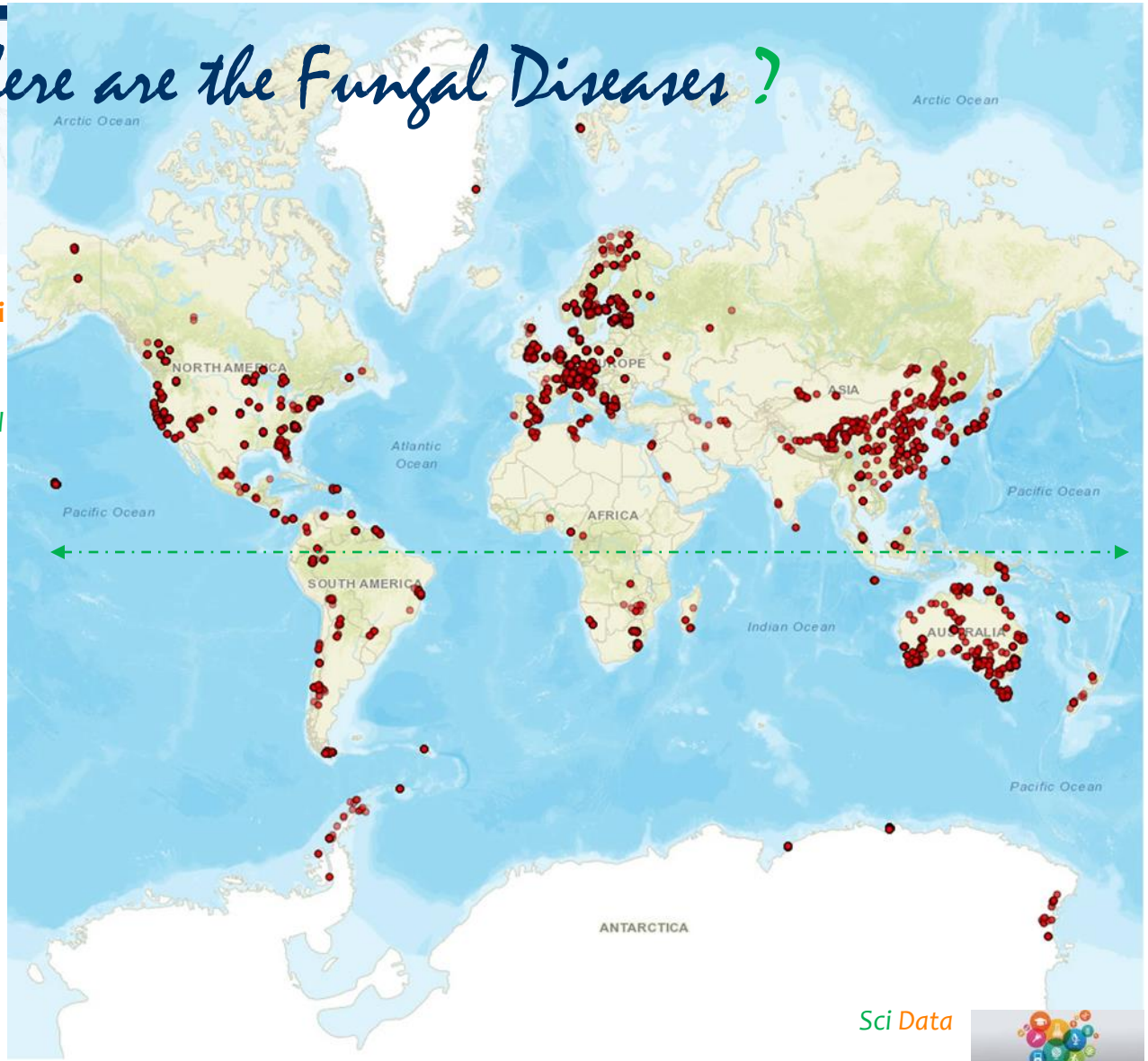
- . . . And then there were MORE



Source: Sanguinetti,M., Posteraro,B., Beigelman-Aubry,C., et.al.; **Diagnosis and treatment of invasive fungal infections: looking ahead**
J Antimicrob Chemother. 2019 Mar 1;74(Suppl 2):ii27-ii37

Where are the Fungal Diseases ?

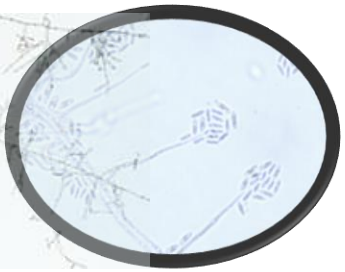
- Map of locations of samples contained in the **Global Fungi Database**
- Each point represents one or several samples where *fungal community composition* was reported using high-throughput-sequencing methods targeting the ITS1 (**i**nternal **t**ranscribed **s**pacer #**1**) or ITS2 marker of fungi
- The GlobalFungi database contains over **600 million observations of fungal sequences** across 17,000+ samples with geographical locations and additional metadata contained in 178 original studies with millions of unique nucleotide sequences of ITS1 or ITS2



Sci Data

Source: Vetrovsky, T., Morais,D., Kohout,P., et.al.; **GlobalFungi, a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies;** *Scientific Data* **; 7(1) :**228 (2020)

WHO Fungal Priority Pathogens



- **Infectious diseases** are among the top causes of **mortality** and a leading cause of **disability** worldwide
- Drug-resistant bacterial infections are estimated to **directly cause 1.27 million** deaths and to **contribute to** approximately **4.95 million** deaths every year, with the greatest burden in resource- limited settings
- Against the backdrop of this major global health threat, Invasive Fungal Diseases (IFDs) are rising overall and particularly among immunocompromised populations
  - The diagnosis and treatment of IFDs are challenged by limited access to quality diagnostics and treatment as well as emergence of antifungal resistance in many settings
- Despite the growing concern, fungal infections receive very little attention and resources, leading to a paucity of quality data on fungal disease distribution and antifungal resistance patterns.
  - Consequently, it is impossible to estimate their exact burden
- In 2017, WHO developed its first bacterial priority pathogens list (WHO **BPPL**)
- WHO has now (10/22/22) developed the **first** **F**ungal **p**riority **p**athogens **l**ist (WHO **FPPL**)
  - This is the first global effort to **systematically prioritize fungal pathogens**, considering their unmet R&D needs and perceived public health importance
  - The WHO FPPL aims to focus and **drive further research and policy** interventions to strengthen the global response to fungal infections and antifungal resistance
  - The development of the list followed a multicriteria decision analysis (MCDA) approach

Source: *WHO fungal priority pathogens list to guide research, development and public health action*. Geneva: World Health Organization; 2022. License: CC BY-NC-SA 3.0 IGO

Prioritization Criteria

- They differ from the other kingdoms;
  - Unlike animals, they have cell walls
  - Unlike plants, they cannot make their own food
  - Unlike bacteria, they hold their DNA within a nucleus and pack cells with organelles—features that make them, at the cellular level, weirdly similar to us . . . . .
- Fungi;
  - Break rocks
  - Nourish plants
  - Seed clouds
  - Cloak our skin
  - Pack our guts
- How much of this are we really familiar with ?
- Our mutual coexistence is now tipping out of balance;
  - Surging beyond the **climate zones** they long lived in
  - **Adapting to environments** that would once have been inimical (obstruct, harm, block)
  - Learning new behaviors that let them leap **between species** in novel ways
  - Becoming **more successful** pathogens

Criterion	Definition/description	Level value
Deaths	Average case fatality rate	**Low:** < 30% **Medium:** 30–70% fatality **High:** > 70% **Unknown:** no reliable data
Annual incidence	Number of new cases per million population each year	**Low:** < 2/million **Medium:** 2–50/million **High:** > 50/million **Unknown:** no data available
Current global distribution	Extent of geographic distribution across the globe	**Localized** in ≤ 2 WHO regions **Globally distributed** in ≥ 3 WHO regions **Unknown:** due to inadequate data
Trends in last 10 years	Evidence of change in incidence/prevalence patterns	**Stable:** no evidence of increasing incidence/prevalence **Increasing:** evidence of increasing incidence/prevalence **Unknown:** due to inadequate data
Inpatient care	Average length of hospital stay required for treatment following initial diagnosis	**Low:** < 2 days **Medium:** 2 days to 2 weeks **High:** > 2 weeks **Unknown:** no data available
Complications and sequelae	Proportion of patients suffering long-term complications of disease	**Low:** expected to affect a minority of patients (e.g. < 10%). **Medium:** expected to affect a significant proportion of patients (e.g. 10–50%). **High:** expected to affect the majority of patients (e.g. > 50%).
Antifungal resistance	Rate (or level) of acquired or intrinsic resistance to antifungal treatment	**Low:** < 10% acquired or intrinsic resistance for all four classes of antifungals. **Medium:** acquired or intrinsic resistance (> 10%) described for agents from one to two classes of antifungals. **High:** acquired or intrinsic resistance (> 10%) described for agents from three to four classes of antifungals. **Unknown:** no reliable data available
Preventability	Transmission/ acquisition dynamics and availability of evidence-based, effective preventive measures	**Low:** transmission/acquisition dynamics well described, and preventive measures ineffective or of low-quality evidence, and/or not widely available or difficult to implement. **Medium:** transmission/acquisition dynamics are not well described, but preventive measures based on moderate or high-quality evidence are available and effective. **High:** transmission/acquisition dynamics are well described, and preventive measures based on moderate or high-quality evidence are universally available and effective. **Unknown:** transmission/acquisition dynamics not well described. No preventive measures described.
Access to diagnostic tests	Availability of diagnostics	**Low:** diagnostics are not available in reference laboratories. **Medium:** diagnostics are available in institutional or reference laboratories but not universally available due to cost, distribution or technical issues. **High:** diagnostics are available and have been successfully implemented in institutional diagnostic laboratories, in at least one but not all high-burden/low-resource settings where disease occurs. **Very high:** diagnostics are universally available in institutional diagnostic laboratories where disease occurs.
Evidence-based treatments	Treatment options are evidence based and accessible	**Very low:** treatment based on expert opinion with limited evidence. **Low:** peer-reviewed, high-quality guidelines available, but first-line treatment options are unaffordable, toxic or unavailable where disease occurs. **Medium:** peer-reviewed, high-quality guidelines with at least one first-line treatment option which is affordable, non-toxic and available where disease occurs. **High:** peer-reviewed, high-quality guidelines with at least one first-line treatment option which is affordable, nontoxic and available where disease occurs, and includes specific recommendations for all main host groups, including paediatrics.

WHO: World Health Organization.

WHO Fungal Priority Pathogens

- The prioritization process focused on fungal pathogens that can cause **invasive, acute** and **subacute** systemic fungal infections ***for which drug resistance or other treatment and management challenges exist***
- The pathogens included were ranked, then categorized into three priority groups (critical, high, and medium)
  - The **critical group** includes;
    - *Cryptococcus neoformans*, *Candida auris*, *Aspergillus fumigatus* and *Candida albicans*
  - The **high group** includes;
    - *Nakaseomyces glabrata* (*Candida glabrata*), *Histoplasma* spp., eumycetoma causative agents, Mucorales, *Fusarium* spp., *Candida tropicalis* and *Candida parapsilosis*
  - The **medium group** includes;
    - *Scedosporium* spp., *Lomentospora prolificans*, *Coccidioides* spp., *Pichia kudriavzeveii* (*Candida krusei*), *Cryptococcus gattii*, *Talaromyces marneffei* (*Penicillium marneffei*), *Pneumocystis jirovecii* and *Paracoccidioides* spp.
- Covered in today's presentation

Source: *WHO fungal priority pathogens list to guide research, development and public health action*. Geneva: World Health Organization; 2022. License: CC BY-NC-SA 3.0 IGO

Medical Encounters

- Hospital
- OPD
- ** Estimate suppressed because of small numbers

	Hospitalizations (2014)	Outpatient visits (2005–2014 average)	Direct medical costs (2019)	Indirect costs (2019)
Aspergillosis	14,820	**	$1.3B	$485M
Blastomycosis	950	**	$24M	$49M
Candida infection				
Invasive candidiasis	12,770	**	$1.2B	$522M
Non-invasive candidiasis	13,990	3,639,037	$2.1B	$443M
Coccidioidomycosis	6,670	**	$204M	$181M
Cryptococcosis	4,755	**	$265M	$269M
Dermatophytosis (ringworm)	690	4,981,444	$845M	$339M
Histoplasmosis	4,630	79,993	$222M	$110M
Pneumocystis pneumonia	10,590	**	$489M	$355M
Mucormycosis	1,140	**	$129M	$131M
Other and **unspecified** fungal diseases	7,355	222,523	$897M	$1.2B

Note: Almost all ~ ½ *$Billion*

Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

Deaths

- 2018-2021
- U.S.
- *National Vital Statistics System Multiple Cause of Death Database*

	2018	2019	2020		2021	
			All	COVID-19–associated	All	COVID-19–associated
Aspergillus	795	723	918	170	1,236	498
Candida	1,010	1,171	1,439	281	1,769	495
Coccidioides	253	192	319	33	359	71
Cryptococcus	290	334	341	24	342	49
Histoplasma	146	133	130	6	199	21
Mucorales spp.	151	134	169	17	232	47
Pneumocystis	371	436	381	13	449	48
Other specified pathogens	116	118	131	3	131	9
Unspecified	1,649	1,623	2,135	362	2,538	746
All	4,746	4,833	5,922	901	7,199	1,967

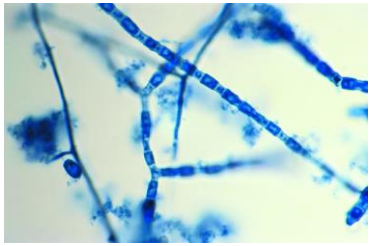
= 50% COVID Total

Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

. . . . Feral Cats of Rio de Janeiro

- Summer, 1998
- 100s cats became ill and died
  - weeping sores on their paws and ears, clouded swollen eyes, what looked like tumors blooming out of their faces
- Then kids, parents
  - Round, crusty-edge wounds opened on their hands, and hard red lumps trailed up their arms as though following a track
  - Eventually 12,000 humans
  - Paraguay, Argentina, Bolivia, Colombia and Panama
  - Rats → Cats → Humans
- Cause: ***Sporothrix brasiliensis***

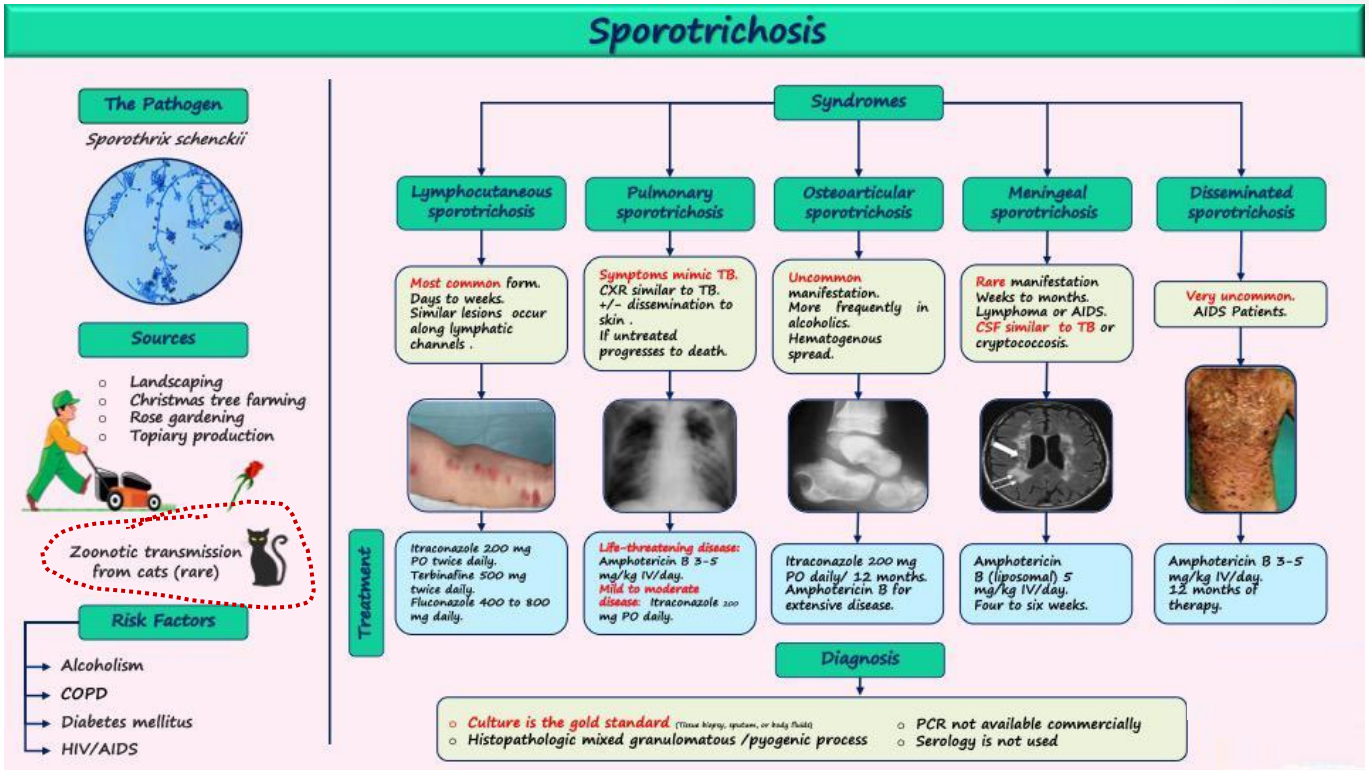
- September, 2018
- 44 y.o. ♂, Patterson, California
  - New house, warehouse manager
  - Seemed like he had a "cold"
  - Nyquil for weeks
- Initial Dx = "Pneumonia"
  - Antibiotics, sent home, use OTCs
  - 280 Lb. → 150, 25% lung capacity
  - Soil → Arthrospores → Humans
  - "Valley Fever" = 8X more common than 2000
  - Wet soil (no problem) → drought (problem)
  - Now found in Washington (2010)
- Cause: ***Coccidioides immitis***, ***Coccidioides posadasii***



Source: "***Deadly Kingdom***" ; Scientific American 324, 6, 26-35 (June 2021); doi:10.1038/scientificamerican0621-26

Increased Opportunities

- **Clinical Disease Diversity**



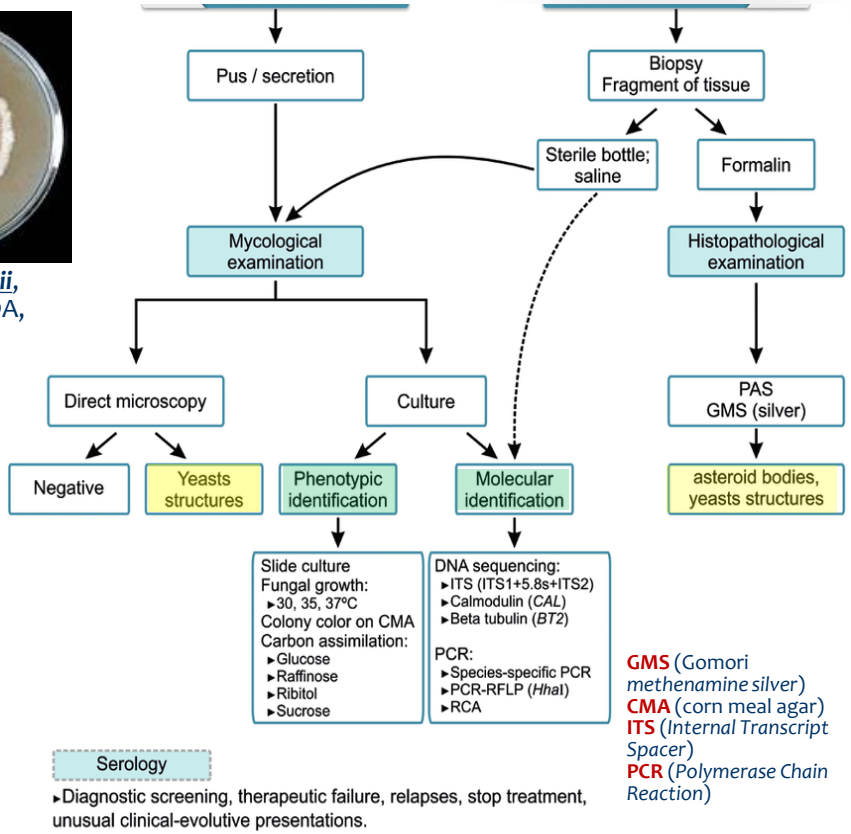
Source: GrepMed ; Diaz , G.; IG: https://www.instagram.com/grepmed/

Increased Opportunities

Clinical Laboratory Diagnostics

- Culture Isolation (Gold Standard)
  - Skin Lesions
  - Biopsy
  - Floating abscess aspiration
  - Sputum, Pus, CNS Fluid, Synovial Fluid
- Direct Microscopy (KOH / Dimethyl Sulfoxide) ??
  - Giemsa makes this better
- Phenotypic biochemicals
  - Thermotolerance, PDA, glucose, raffinose, ribitol, and sucrose
- Histopathology
  - Generally poor (*not enough fungal elements in the tissues*)
- Serology
  - ELISA (SsCBF [*Sporothix schenckii* Con A-Binding Fraction])
- Molecular
  - PCR, PCR-RFLP, Species-specific PCR, Rolling Circle Amplification (RCA)

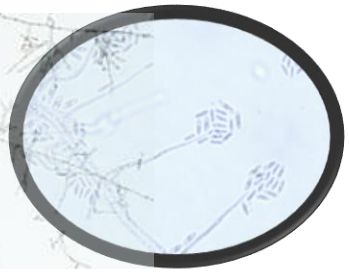
Sporo. schenckii, Mold-phase, SDA, 6 da., 30°C



GMS (Gomori *methenamine silver*)
CMA (corn meal agar)
ITS (*Internal Transcript Spacer*)
PCR (*Polymerase Chain Reaction*)

Source: Orofino-Costa, R, deMacedo, M.P., Rodrigues, A.M., et.al; Anais Brasileiros de Dermatologia · July 2017 DOI: 10.1590/abd1806-4841.2017279

Where are the Fungal Diseases ?



- **H_1N_1 Avian Influenza, 2009**
- Netherlands
  - **Flu** patients arrive at ER unable to breath, going into shock, died in days
  - By 2018 "Invasive Pulmonary Aspergillosis" = 1/3 Flu Pts, 2/3 died
- Then **SARS CoV-2**, 2020
  - China, France, Belgium, Germany, the Netherlands, Austria, Ireland, Italy and Iran
  - Worse than *Can. auris* because *Aspergillis* cannot be contained, its just out there everywhere
  - Environment → Compromised Lungs → Humans
- Cause: ***Aspergillis fumigatus***

Note: Invasive Aspergillosis (U.S.) ↑ 3% per year, 2000- 2013 [*now costs $1.2 Billion/ year*] 180 spp in total, *flavus*, *fumigatus* and *terreus* = most common

- Most patients have an effect in the respiratory system, but their signs and severity vary greatly
- Aspergillus, is ubiquitous — indoors and outdoors, most strains are harmless, but a few can cause serious illnesses when people with weakened immune systems, underlying lung disease or asthma inhale the spores
- In some, the spores trigger an allergic reaction, others develop mild to serious lung infections
- Worst = invasive aspergillosis — CVS and beyond
- NOT a "reportable" disease in the U.S.
- Rx:
  - Observation - Probably WAY more common
  - Antifungals - Case load probably better recognized
  - Surgery - Rare
- Allergic bronchopulmonary aspergillosis (ABPA) = 1 in 15 **cystic fibrosis patients**, 2.5% of adults who have asthma also have ABPA, which is approximately 4.8 million people worldwide

Source: Vallabhaneni S, Benedict K, Derado G, Mody RK. *Trends in hospitalizations related to invasive aspergillosis and mucormycosis in the United States, 2000-2013external icon*. Open Forum Infect Dis. 2017 Winter;4(1):ofw268

Critical

Clinical Laboratory Diagnostics

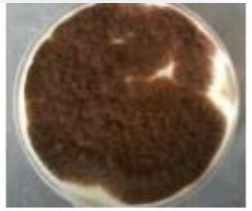
Aspergillus fumigatus

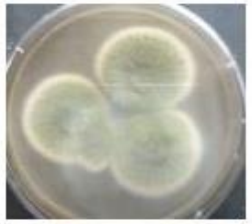
Common pathogens

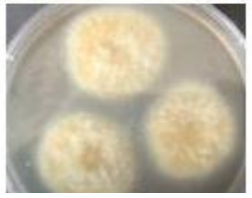
- At least **5** others
- Total = **180** spp.
- No more than **39** spp. have ever been isolated from humans

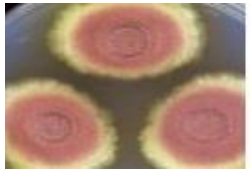
Species Complex

Species	Conidiophore length (mm)	Vesicle width (µm)	Phialides	Conidia	
				Diameter (µm)	Color
Aspergillus niger *	1.5-3.0	45-75	Biseriate	4.5-5.0	Black
Aspergillus fumigatus *	<0.3	20-30 i.e. Only top half conidiogenous	Uniseriate	2.5-3.0	Green or bluish green
Aspergillus flavus *	<1	25-45	Uniseriate or biseriate	3.5-4.5	Yellow to green
Aspergillus terreus *	<0.25	30-50	Biseriate Compactly columnar	1.5-2.5	Cinnamon-buff to sand brown in colour with a yellow to deep dirty brown reverse









* = Species Complex
(5 others in Clinical Micro.)
(***Asp.*** **versicolor**, ***ustus***, ***tanneri***, ***nidulans***, ***terreus***)

Source: "***Deadly Kingdom***"; Scientific American 324, 6, 26-35 (June 2021); doi:10.1038/scientificamerican0621-26

Critical

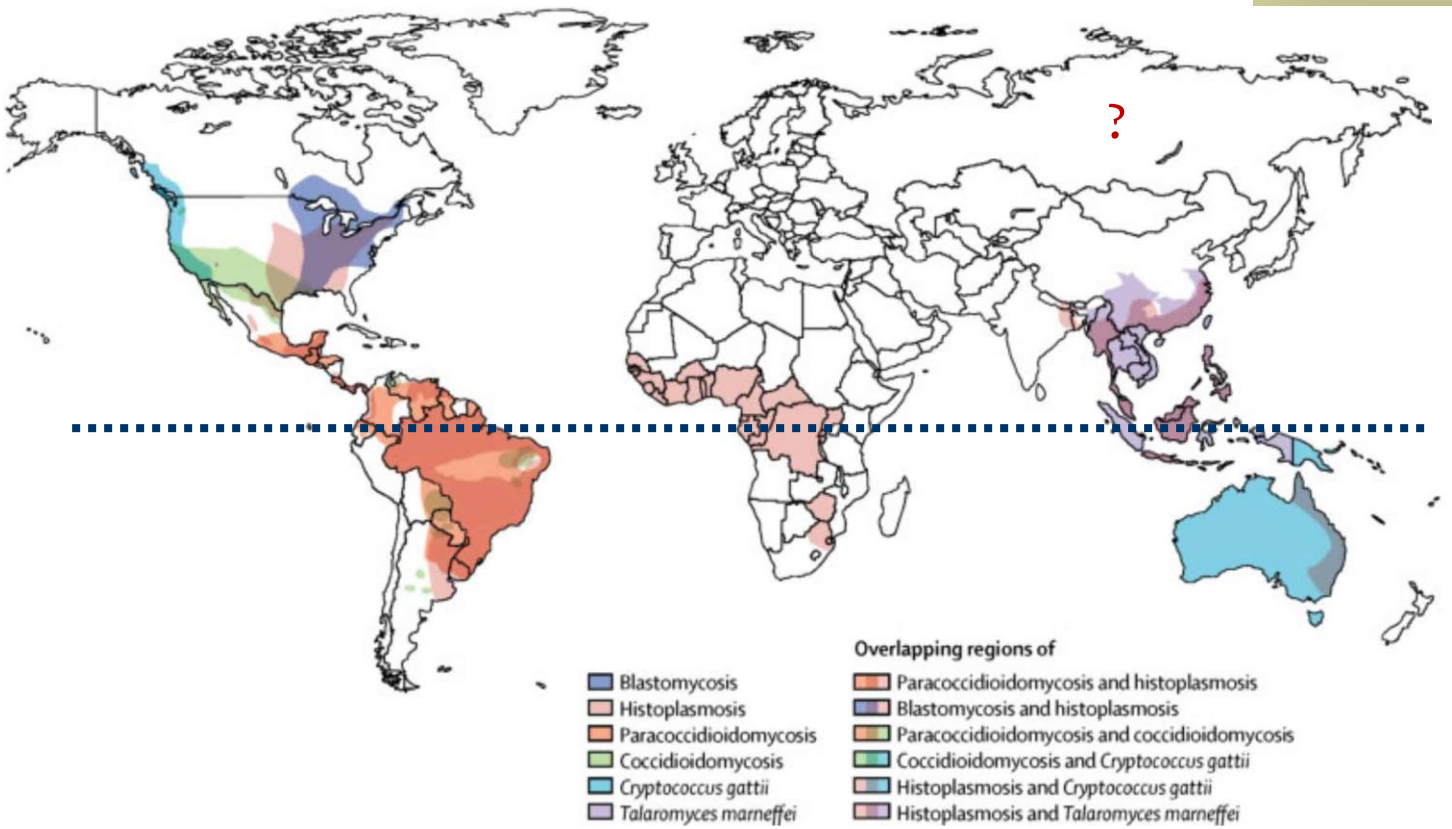
Clinical Laboratory Diagnostics

Aspergillus fumigatus

- Wet mounts of clinical specimens or culture;
  - Gram stain
  - Conventional histopathology, provide clues that suggest the presence of *Aspergillus* spp.
- Tissue;
  - Blankophor or Calcofluor mixed with 10%–20% potassium hydroxide (KOH)
    - = Stains fungal cell walls and improves detection of fungi
    - Calcofluor crystallizes in an alkaline pH, Blankophor does not and it can be stored in a working solution for up to a year
    - Phenotypic markers detected by histopathologic stains
- Confirmation of microscopic findings by culture is always desirable and, in most cases involving opportunistic molds, essential for definitive identification of the pathogen
  - In one case of *Aspergillus niger* sinusitis, *Asp. niger* conidia were confused with the yeast cells of *Candida* spp. and cross sections of the stipes of *Asp. niger* were confused with the broad hyphae of a zygomycete
- Don't forget other aspergilli associated with invasive aspergillosis
  - *Asp. flavus*, *Asp. niger*, *Asp. nidulans*, and *Asp. terreus* have similar growth (malt extract agar and Czapek yeast agar after incubation for seven days at both 25°C and 37°C)
- Drug resistance of some *Aspergillus* spp. is a threat, full identification, not only of *Asp. fumigatus*, but also of the less commonly isolated species, is needed
- Remember to recognize atypical isolates of *Aspergillus* spp.
  - Poorly sporulating (white) strains of *Asp. fumigatus* with decreased susceptibilities to several antifungal drugs have been reported recently

Source: McClenny,N.; ***Laboratory detection and identification of Aspergillus species by microscopic observation and culture: the traditional approach;*** *Medical Mycology*, Volume 43, Issue Supplement_1, January 2005, Pages S125–S128

CNS Fungal Infections of Humans



Source: Geographic distribution of central nervous system fungal infections caused by endemic fungi. (Reproduced with permission from Elsevier: Schwartz et al.)

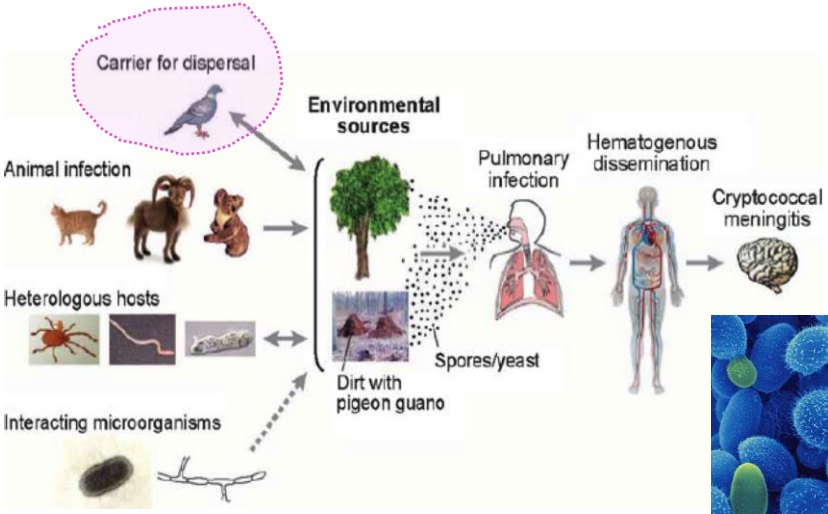
Critical

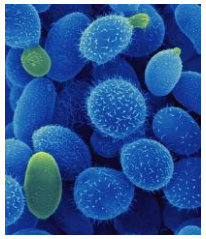
Cryptococcus neoformans

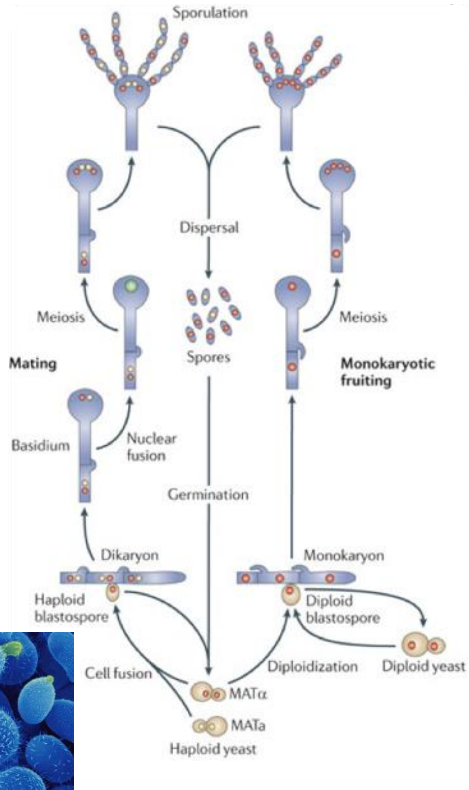
Species Complex

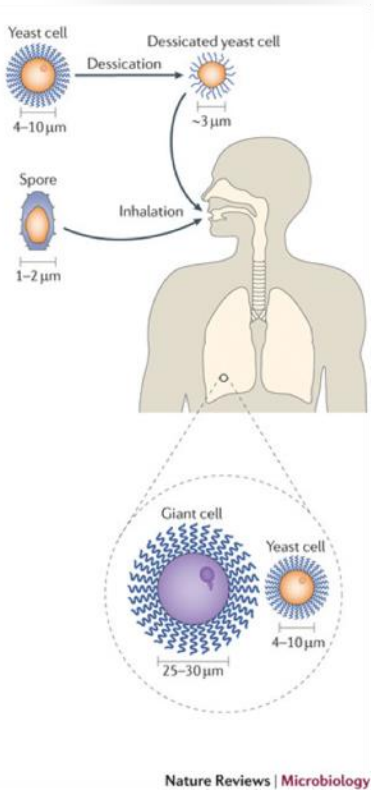
Cryptococcus neoformans *(Species Complex) – next slide*

- ***Cryptococcus neoformans*** = environmental (soil, trees), primarily tropical and sub-tropical, some temperate (British Columbia, U.S.)
- Lungs, central nervous system (cryptococcal meningitis), etc.
- Humans and animals in California since 1960s
- Pigeon droppings (most common source)









Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

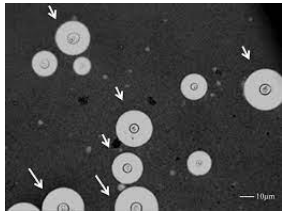
Cryptococcus neoformans

Critical

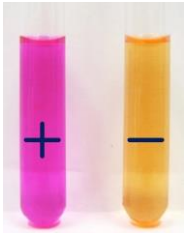
Clinical Laboratory Diagnostics

Cryptococcus neoformans

- Clinical **laboratory testing** algorithm;
  - Urease test
  - Culture isolates grown on IMA agar
  - Remel rapid urea broth
  - CGB medium
  - IGS sequence analysis
- **Virulence of Markers**
  - Polysaccharide capsule
  - Phenoloxides
  - Growth rate at 37°C



India Ink Mx



Urease test

(-) Birdseed Agar (+)

Crypto. neoformans Species Complex (*C. neoformans sensu stricto* (formerly *C. neo.* var *grubii* [serotype A]) and *C. deneoformans* (formerly *C. neoformans* var *neoformans*[serotype D])

Staib 1962 *Guizotia abyssinica* seeds, 1966, Shields and Ajello modified Staib's (antibiotic), now = **Caffeic Acid Agar** or Niger Seed Agar

Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

Medium

Cryptococcus gattii

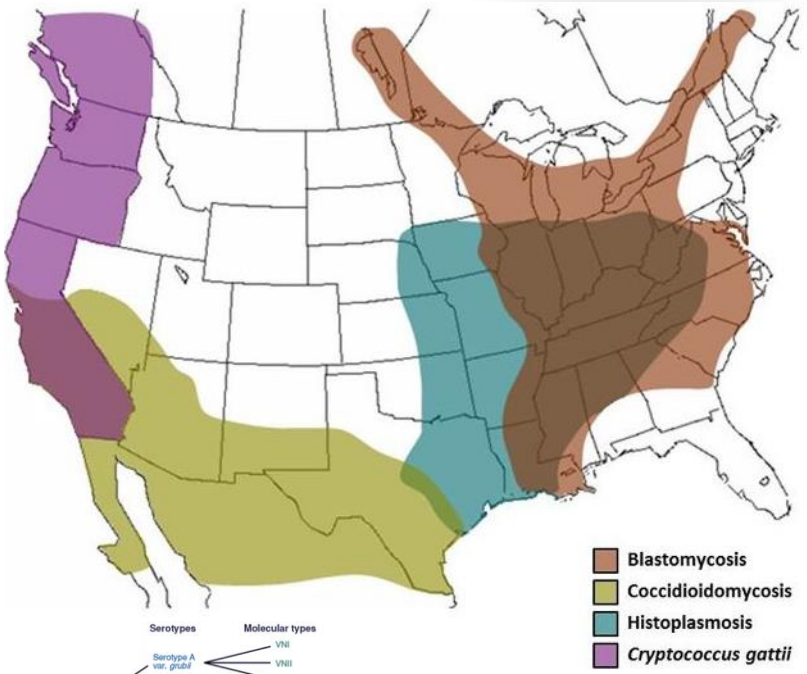
Species Complex

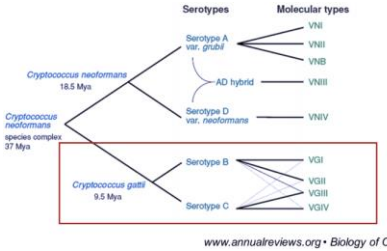
Cryptococcus gattii *(Species Complex)*

(***Crypto***. ***neoformans*** serotypes B and C)

- ***Cryptococcus gattii*** = environmental (soil, trees), primarily tropical and sub-tropical, some temperate (British Columbia, U.S.)
- Lungs, central nervous system (cryptococcal meningitis), etc.
- Humans and animals in California since **1960**s
- Until the past few decades, *Crypto. gattii* was not known to cause locally acquired infections elsewhere in the United States;
  - **2004** = different strains of ***Crypto. gattii*** causing illness in the Pacific Northwest (Oregon and Washington)
  - **2001** = Canada (Water, Soil)
  - **2007** = Southeast (no travel to the West Coast)
  - Australia; Papua New Guinea; British Columbia, Canada; and the U.S. Pacific Northwest, overall mortality rate =13% to 33%
  - Tree hollows (variety) → animals → etc.

Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***





www.annualreviews.org • Biology of Cryptococcus

Crypto. ***gatt***i species complex: *C. bacillisporus*, *C. decagattii*, *C. deuterogatti*, *C. gattii sensu stricto*, *C. tetragattii*

CDC

Medium

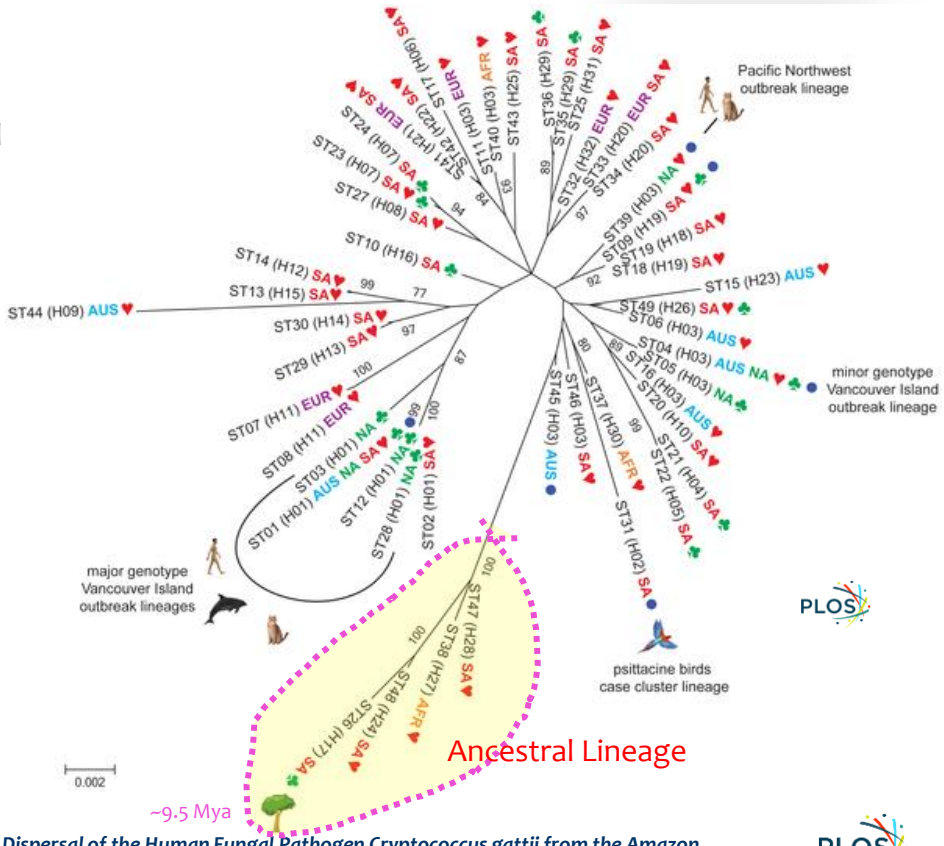
Cryptococcus gattii

Species Complex

Cryptococcus gattii (Species Complex)

(***Crypto. neoformans*** serotypes B and C)

- Genetic diversity of ***Crypto. gattii*** genotypes as assessed by SCAR-MLST, microsatellite analysis and AFLP fingerprint analysis
- Ancestral lineage (H17, H24, H27, H28)
  - Sub-populations =
  - Africa (AFR)
  - Australia (AUS)
  - Europe (EUR)
  - North America (NA)
  - South America(SA)
- (Clinical)
- Environmental (♣)
- Veterinary (•)
- Highly distributed (❤)



Source: Hagen F, Ceresini PC, Polacheck I, Ma H, Nieuwerburgh Fv, et al. (2013) ***Ancient Dispersal of the Human Fungal Pathogen Cryptococcus gattii from the Amazon Rainforest***. PLOS ONE 8(8): e71148

Cryptococcus gattii

Medium

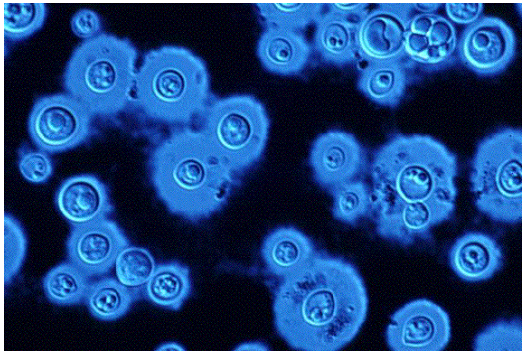
Clinical Laboratory Diagnostics

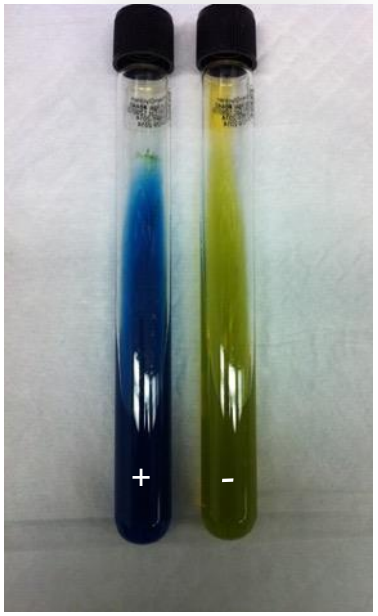
Species Complex

Cryptococcus gattii (Species Complex)

(*Crypto. neoformans* serotypes B and C)

- Tissue, body fluid (blood, CSF, sputum)
  - Microscopic
  - Antigen test
  - Culture (only way to distinguish)
    - Canavanine-Glycine-Bromthymol blue (CGB) agar
    - MALDI-TOF can 'sorta do this as well
  - Chest x-ray , CT scan (lungs, brain, etc.)





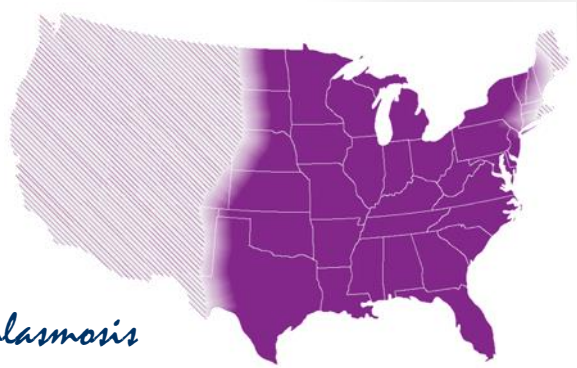
Crypto. gattii (left) on CGB agar

Note: May take 2-5 da.

Source: Centers for Disease Control and Prevention; *Mycotic Disease Branch*

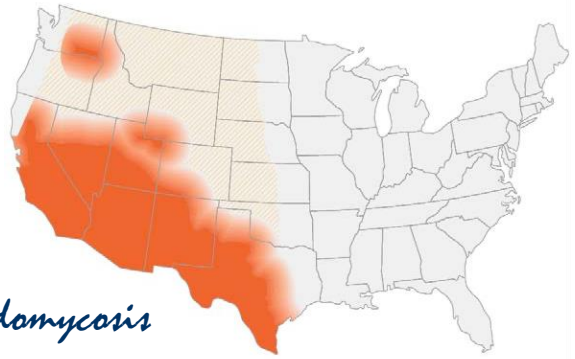
The Dimorphs

- Each disease is NOT distributed evenly in the shaded areas
- Each might not be present everywhere in the shaded areas
- Each can also be outside the shaded areas



Histoplasmosis

Blastomycosis



Coccidiodomycosis

Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

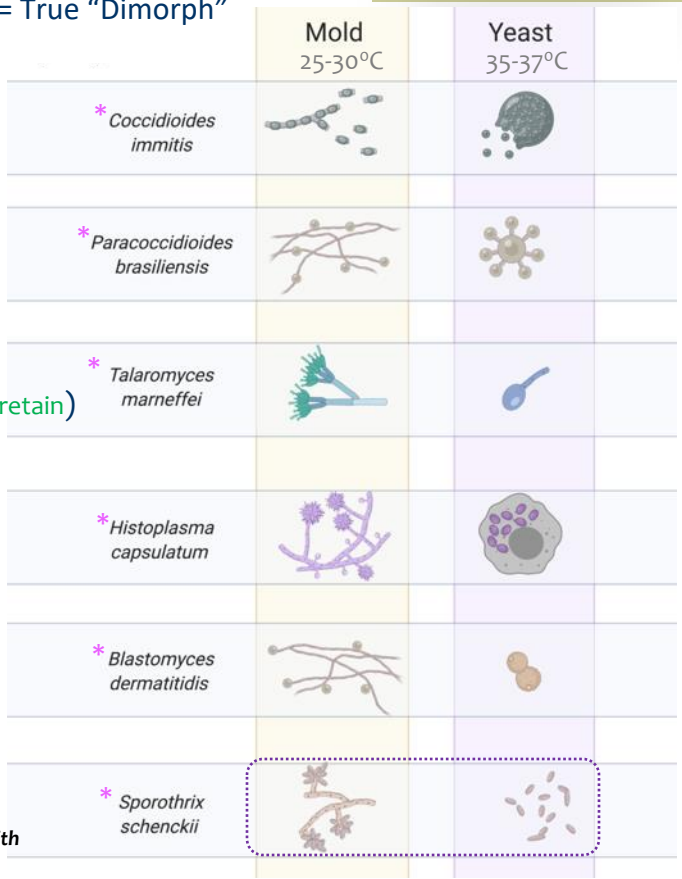
The Dimorphs

Note: ***Cokeromyces recurvatus***, also a thermal dimorph, but is a Mucormycete

* = True "Dimorph"

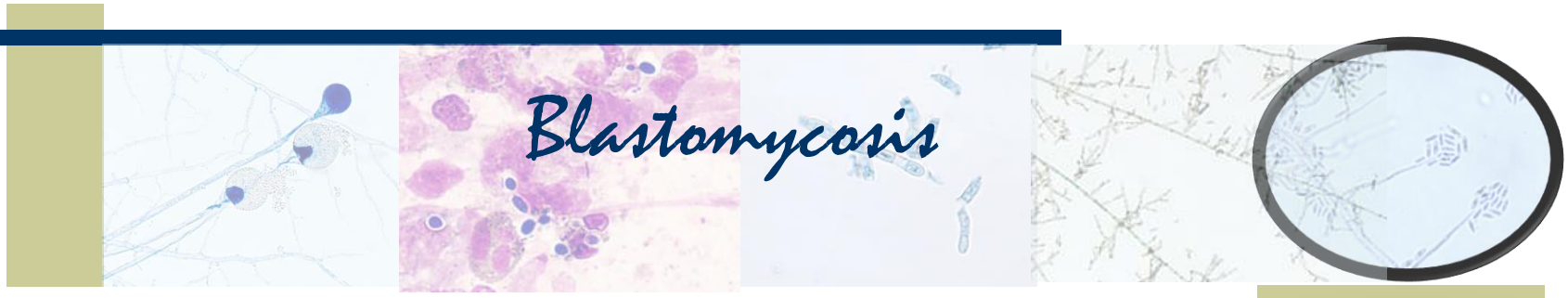
- The clinically significant thermal dimorphs are;
  - *Blastomyces dermatiditis* (1894, Gilchrist)
  - *Coccidiodes immitis / posadasii* (1891, Posadas)
  - *Histoplasma capsulatum* (1906, Darling)
  - *Paracoccidiodes brasiliensis / lutzii* (1904, "Brazil")
  - *Sporothrix schenckii* (Nutritional Dimorph) (1896, Schenck)
    - Complex of 6 species
  - *Talaromyces marneffei* (*Penicillium marneffei*) (1956, Segretain)

Molecular phylogenetic analysis of dimorphic fungal pathogens. Human pathogens (red typeface), plant pathogens (green typeface), and nonpathogens (**black** typeface) are shown and marked as true dimorphic species with free-living vegetative cell types (blue circle) and species with morphological transitions that are not free-living vegetative cell types (blue triangle). Most are **Ascomycota**



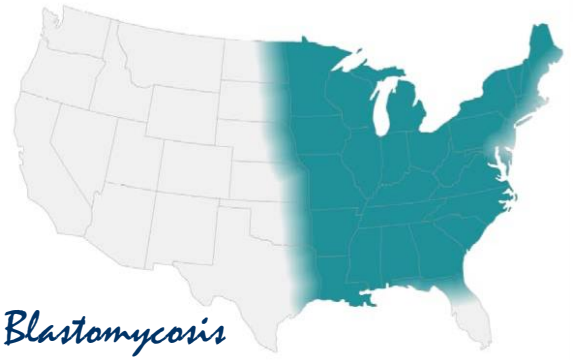
Source: Sil,A, Andrianopoulous, A,; ***Thermally Dimorphic Human Fungal Pathogens—Polyphyletic Pathogens with a Convergent Pathogenicity Trait***; Cold Spring Harb Perspect Med. 2015 Aug; 5(8): a019794

Note: Other "genetically dimorphic spp.,* ***Can. albicans***, ***Can. glabrata***, ***Crypto. neoformans***, ***Trichophyton rubrum***, ***Asper. fumigatus***

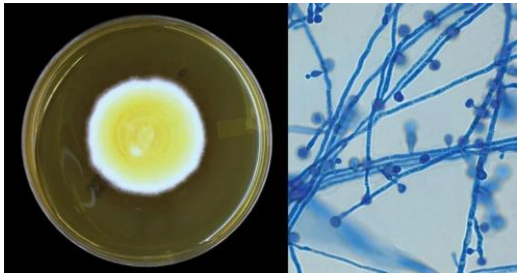


Blastomyces dermatitidis* / *gilchristii (Morphologically indistinguishable)

- Blastomycosis remains poorly understood, and it is important to know the fungi that cause blastomycosis:
  - Are not distributed evenly in the shaded areas. For example, hotspots exist in northern Minnesota and Wisconsin
  - *Sequenced strains*: ***Blast. dermatitidis*** SLH14081 (most virulent) , ER-3, ATCC 18188, and ATCC 26199
- Blastomycosis also occurs in parts of Canada, with hotspots in western Ontario
- New There are also a small number of illnesses caused by a species called ***Blastomyces helicus*** in the western United States and Canada
  - Usually different from the more common type of blastomycosis in eastern North America
- New Recently a small number of illnesses caused by a species called ***Blastomyces percursus*** isolated from Africa / Asia



Note: ***Emmonsia parva*** has been re-named ***Blast. parvus***

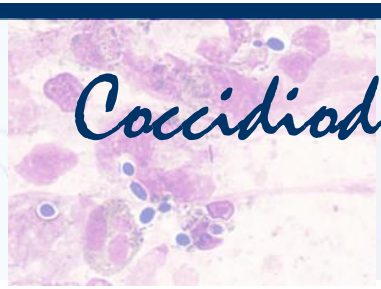


Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

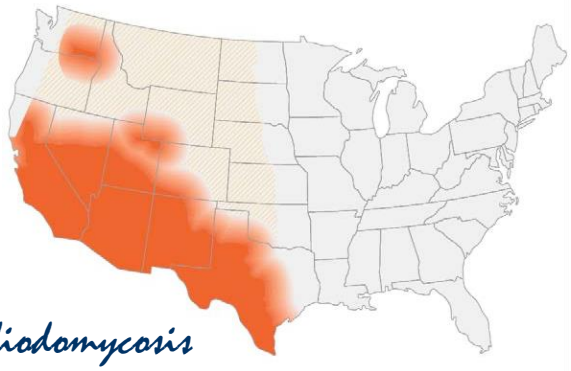
Source: ***Blastomyces dermatitidis***- An Overview; May 29, 2021 by Faith Mokobi

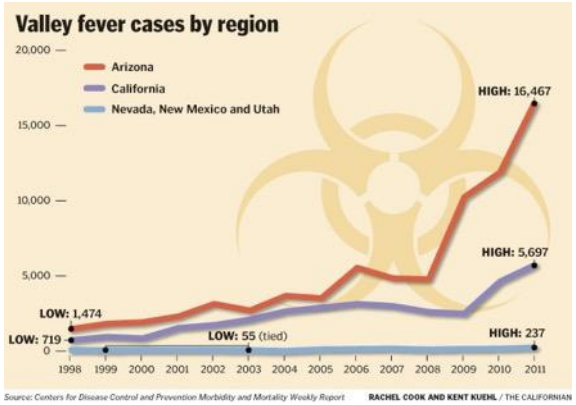
Coccidiodomycosis

High



- CDC's current estimate
  - The causative fungi of Valley fever are ***Coccidioides immitis*** and ***Coccidioides posadasii***
  - ***U.S.*** = ***Cox. immitis*** primarily in California, as well as Washington State
  - ***Cocc. posadasii*** is found primarily in Arizona, as well as New Mexico, Nevada, Utah, Texas, and portions of southern California
- Southern California, particularly the southern San Joaquin Valley, and southern Arizona, including metropolitan Phoenix and Tucson, have the highest reported rates of Valley fever
- Likely also common in parts of West Texas and along the Rio Grande River
- ? north of these areas (as far north as eastern Washington State and the northeast corner of Utah)
- Likely in many western states
- ? ↑ as environmental conditions change





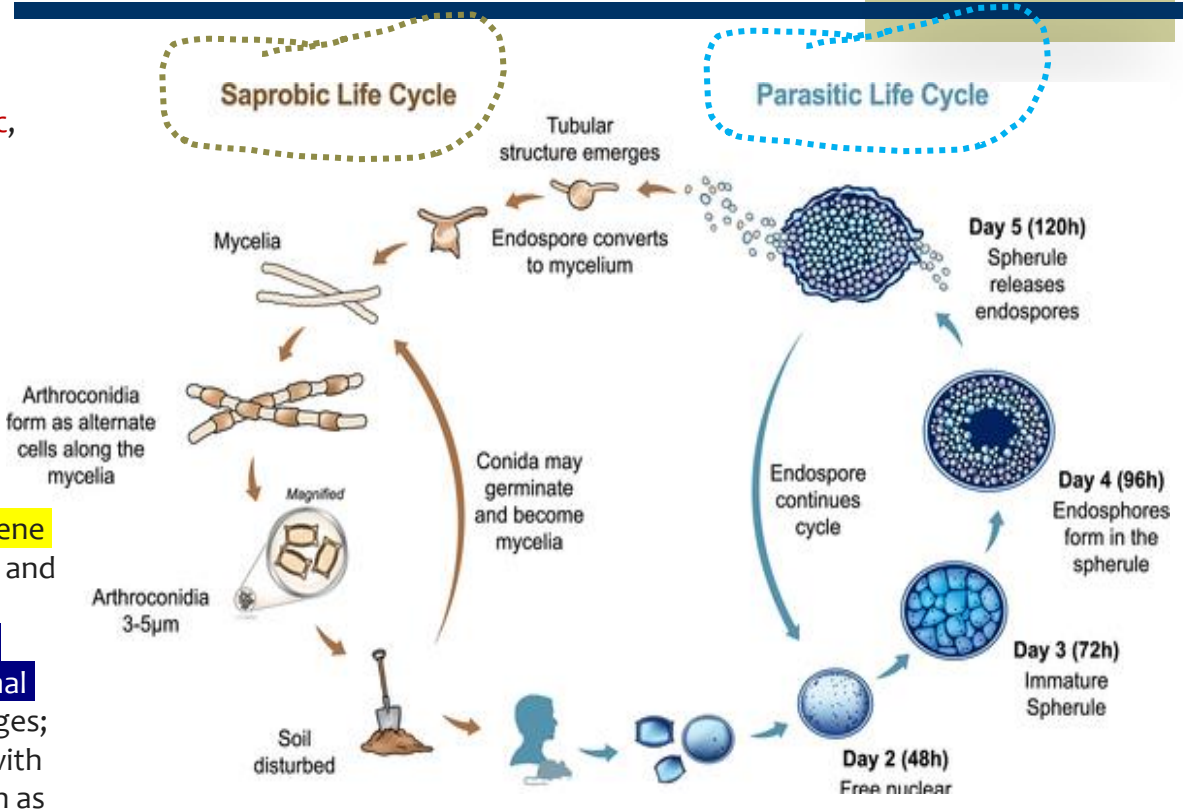
Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

Coccidiodomycosis

High

Clinical Laboratory Diagnostics

- **Coccidioides immitis** & ***Cocc. posadasii*** are pathogenic, dimorphic, soil-dwelling Ascomycetes in the Onygenales order
- On average, both *Coccidioides* species have 29 Mb haploid genomes, containing appx. 10,000 open reading frames (ORFs) on five chromosomes
- *Coccidioides*' most recent common ancestor underwent gene family expansions for proteases and keratinases, membrane biology genes, and toxin production, all likely utilized for survival in animal tissues and morphological changes; and a loss of genes associated with degradation of plant tissue, such as tannases, cellulases, and cutinases



Note: Best ID = DNA Sequencing or MALDI-TOF MS

Source: Lewis ERG, Bowers JR, Barker BM (2015) Dust Devil: The Life and Times of the Fungus That Causes Valley Fever. PLOS Pathogens 11(5): e1004762. https://doi.org/10.1371/journal.ppat.1004762; https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1004762

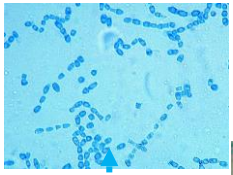
PLOS

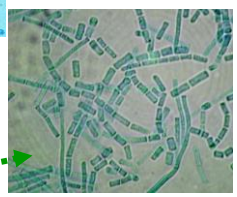
Coccidiodomycosis

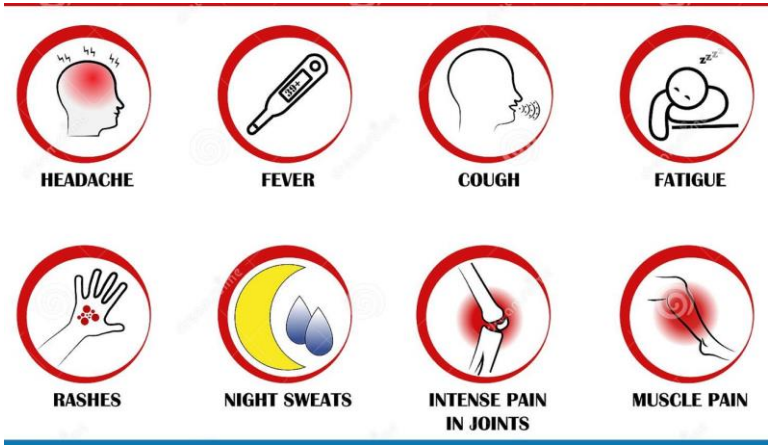
High

Clinical Laboratory Diagnostics

- **Coccidioides immitis** & ***Cocc. posadasii***
- Both are distantly related to other dimorphic human pathogens, such as ***Histoplasma*** *(Ajellomyces)* ***capusulatum***, in the new family Ajellomycetaceae
- Both species have well-characterized asexual life cycles with distinct saprobic and parasitic stages, and only molecular evidence of a sexual cycle
- In the saprobic phase, *Coccidioides* rotate between mycelial and arthroconidial stages
- The most pathogenic strains can cause fatal disease within eight days with as few as 50 arthroconidia administered intranasally in immunocompetent mice
  - minimum dosage is not known in humans, but the infectious dose can be 1 arthroconidium
- Mx: Can be confused with ***Geotrichum candidum*** or ***Malbranchea*** *spp.*





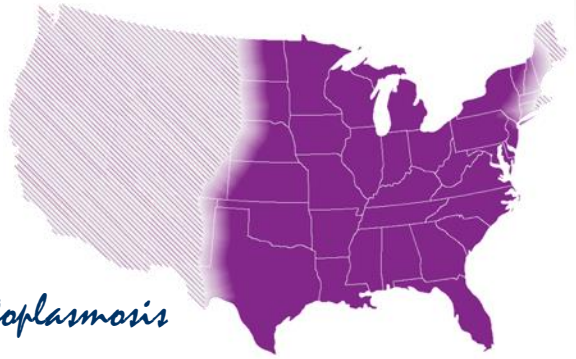


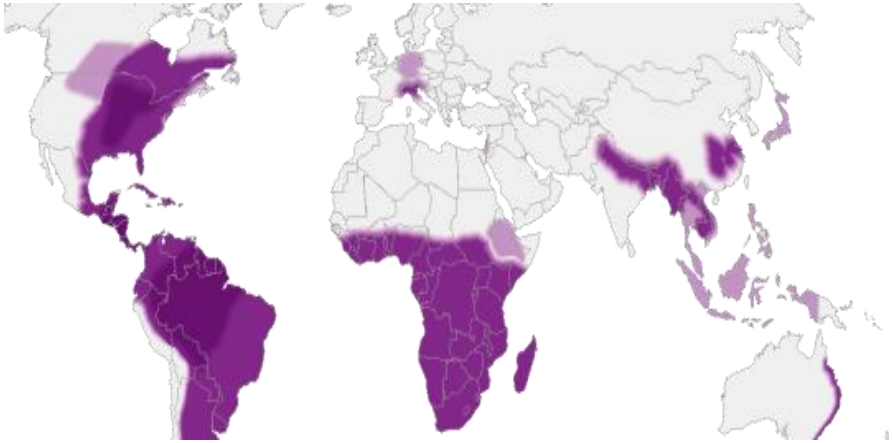
Source: Lewis ERG, Bowers JR, Barker BM (2015) Dust Devil: The Life and Times of the Fungus That Causes Valley Fever. PLOS Pathogens 11(5): e1004762. https://doi.org/10.1371/journal.ppat.1004762; https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1004762

PLOS

Histoplasmosis

- ***Histoplasma capsulatum*, *Hist. dubosii*, *Hist. farciminosum*** (or *H.c.* var dubosii, *H.c* var capsulatum)
- **World-wide**, but most common in *North America* and *Central America, (Hist. dubosii = C/W Africa)*
- U.S. = mainly lives in **soil** in the central and eastern states, particularly areas around the **Ohio and Mississippi River Valleys**
- Also found in parts of Central and South America, Africa, Asia, and Australia
- *Really* thrives in soil or other environmental material containing bird or bat droppings
- Most cases of histoplasmosis are not part of an outbreak
  - Outbreaks linked to a common source occur occasionally, particularly after events that disturb soil or other environmental material contaminated with bird or bat droppings



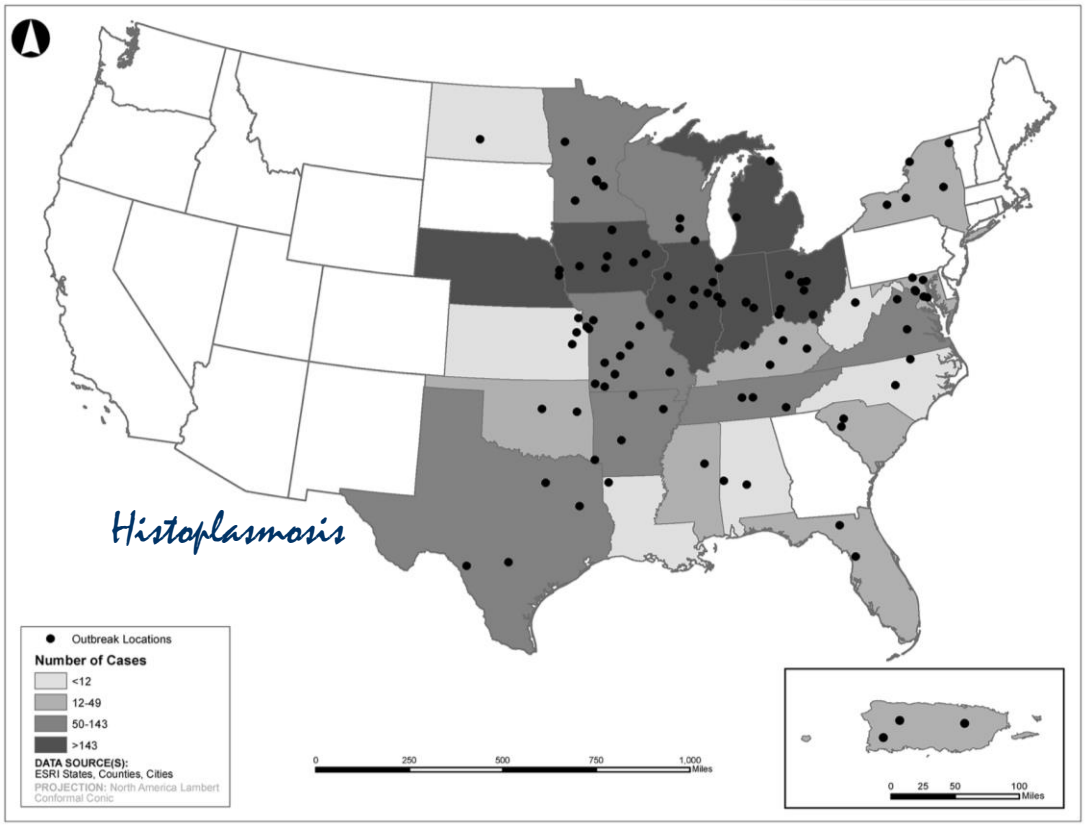


Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

Histoplasmosis

High

- Locations of **105** histoplasmosis outbreaks that happened during **1938–2013** and the number of outbreak-related cases by state or territory.
- Some of these outbreaks happened in places NOT where *Histoplasma* was expected to be found



Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

Histoplasmosis

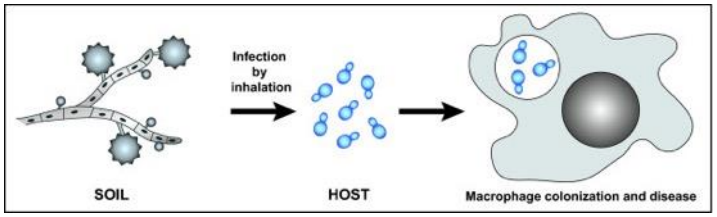
High

Clinical Laboratory Diagnostics

Histoplasma capsulatum var *dubosii*

Histoplasma capsulatum var *capsulatum*

Both thermally dimorphic fungi, existing as hyaline mold form in nature and in culture at 25°C and intercellular budding yeast form in culture at 37°C



- Macroconidia-large spherical, 5-15µm conidiospore
- Microconidia=small elliptical or oval 2-9µm sessile spore
- Histoplasma yeast = 2-4µm oval found intracellularly in target host
- Histoplasmosis is an intracellular mycosis of **reticuloendothelial system**
- It is a systemic infection involving lymphatic tissue, liver, spleen, Kidney, bone marrow and other body parts
- Also known as Cave dweller's disease or Darling disease or Reticuloendothelial cytoplasmosis or Ohio valley disease.
- Infection occurs by **inhalation of dust** contaminated with *Hist. capsulatum* conidia
- Inhaled conidia are phagocytosed by **alveolar macrophage** and Polymorphoneutrophils (PMN)
- Conidia = resistant to oxidative burst and lysosomal fusion (org. captures iron/calcium from macrophage) and behave as intracellular parasite.
- ∴ multiply rapidly inside macrophage and spread throughout the body
- ∴ small inflammatory or granulomatous lesions (lungs and spleen) → calcification

Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

CDC

Histoplasmosis

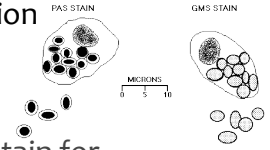
High

Clinical Laboratory Diagnostics

- Specimen: sputum, respiratory secretion, excised skin, biopsy from lymph node and bone marrow, peripheral blood, scrapping from lesion etc

1. **Microscopic examination:**

Mx: ? *Blastomyces spp*, *Sepedonium spp.*



- Giemsa or Wright strain stain preparation
- H&E stain
- PAS stain
- Direct Examination Tissue Specimens stain for fungi – Giemsa, Wright, routine histology - H&E- small yeast (2-4 ?) intracellular in macrophages- Sputum - KOH or calcofluor white

2. **Culture:**

- Sabouraud Dextrose Agar (SDA); Histoplasma form white buffy brown cottony colony with pale yellowish reverse on SDA at 25-30˚
  Mx = Mold (RT) on SDA
- **Hyphal to yeast conversion at 37°C**
  Yeast cells, Yeast-like colonies
- Brain Heart Infusion Agar (BHI) agar: smooth white creamy yellow colored moist yeast like colony appear on BHI agar at 37˚
- Glucose Cysteine Blood agar (GCB), Potato Dextrose Agar (PDA)
- Yeast Extract Agar (YEA),Littman's Oxgall medium

3. **Serology:** Immunodiffusion test, compliment fixation test, ELISA
4. Culture filtrate (histoplasmin) is inoculated to observe cell mediated immunity
   3. Serology = Complement fixation test
   4. Precipitation and agglutination
5. **PCR/ DNA probe, DNA Sequencing**
6. **Histoplasmin Skin test**
7. **Animal Innoculation:** inoculating macroconidia of Histoplasma into a mouse
8. **Histopathological examination**
9. **X-ray examination**
10. **MALDI-TOF MS**

Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

Histoplasmosis

High

Clinical Laboratory Diagnostics

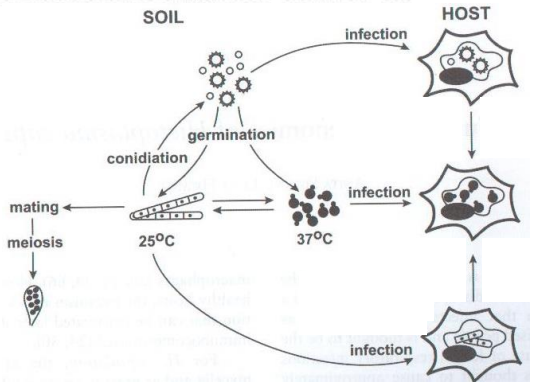
- Dimorphic fungus
  - **Mycelium** at 25-30° C - Sexual multi-cellular saprophyte, septate, form microconidia and macroconidia
  - Yeast at 37° C - Asexual unicellular intracellular parasite, white, thin walled, oval
  - Mycelial form is most commonly found in the environment **Reservoir is soil enriched with droppings of birds or bats**
  - Human, many domestic animals, bats are infected by ingestion of spores
- Infection begins with inhalation of microconidia or hyphal fragments
  - Mycelial form → yeast form Triggered by elevated temperatures and increased cysteine levels
- Yeast cells = phagocytized by host immune system, survive phagocytosis
- Apoptosis of infected macrophages → spread Infection (usually self-limiting) if immunocompetent (cell mediated immunity in 15 days)

Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

***HISTOPLASMA* DIAGNOSTIC TESTING IN CEREBROSPINAL FLUID**

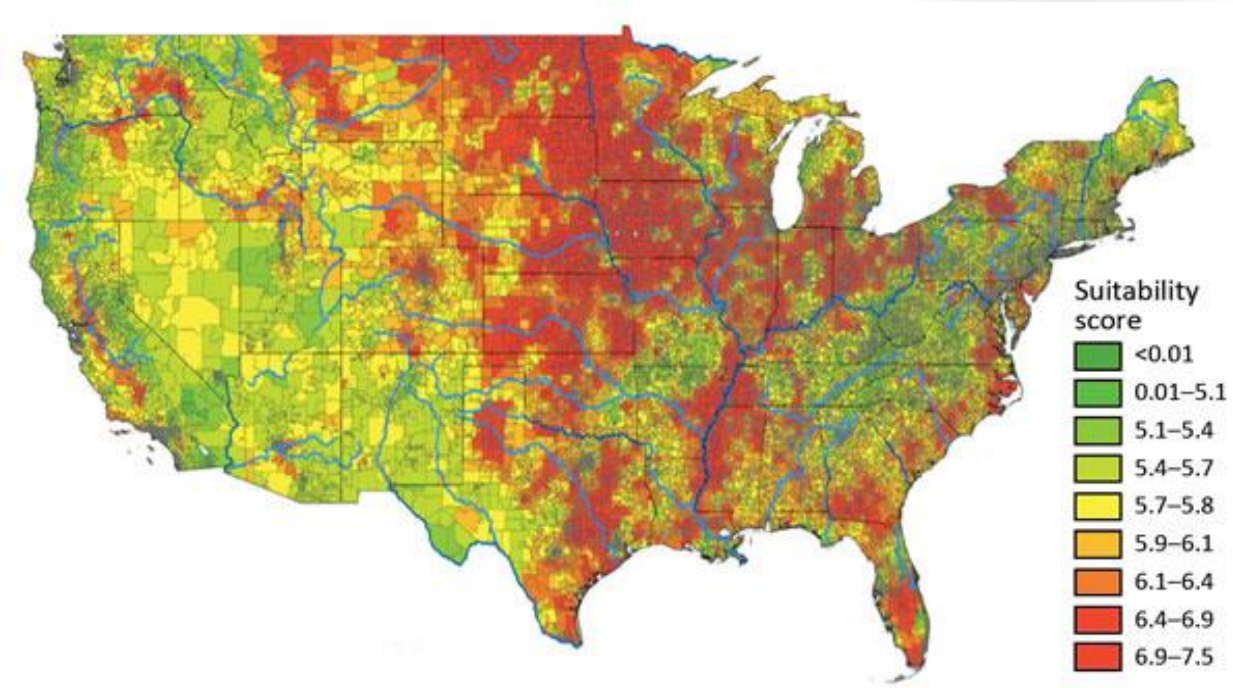
Test	Sensitivity[1]	Specificity[2]	PPV(%)	NPV(%)
Culture	✗ 9/47 (19)	✓ 119/119 (100)	✓ 100	91.8
Antigen	39/50 (78)	140/145 (96)	71.8	97.5
IgG or IgM anitbody	37/45 (82)	142/153 (92)	52.1	97.9
Antigen, IgG or IgM anitbody	✓ 48/49 (98)[3]	✓ 139/153 (90)[4]	54.7	✓ 100
ID antibody	✗ 19/43 (44)	✓ 13/13 (100)	✓ 100	94.1
CF antibody	✗ 5/10 (50)	13/14 (93)	43.9	94.4
Antigen, ID or CF antibody	✓ 44/50 (88)[3]	✓ 139/145 (96)[4]	70.9	98.6

1TRUE POSITIVE/TOTAL (%), 2TRUE NEGATIVE/TOTAL (%), 3P VALUE = 0.121, 4P VALUE = 0.073



Histoplasmosis

- Mean *Histoplasma* **site suitability score** by US ZIP code. Red reflects greater histoplasmosis suitability;
- green = less suitability
- Weighted mean score (Color Table) was calculated for each ZIP code
- Data for geographic regions west of the Rocky Mountains are considered *insufficient* because of limited surface water data

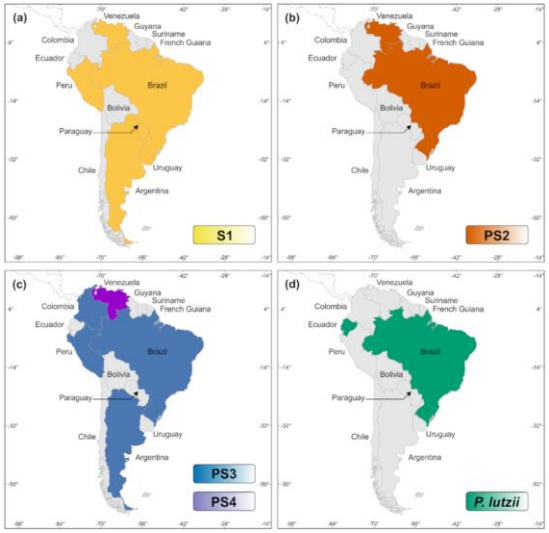


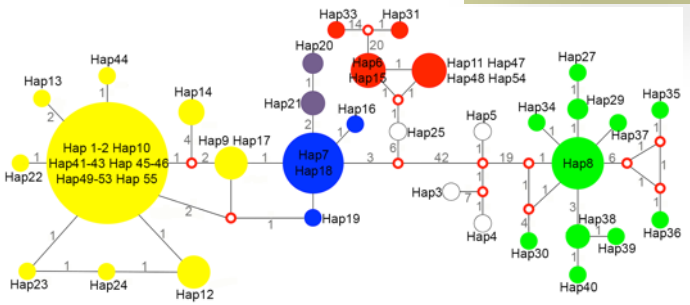
Source: Maiga AW, Deppen S, Scaffidi B, Baddley J, Aldrich MC, Dittus RS, et al. ***Mapping Histoplasma capsulatum Exposure***, United States. Emerg Infect Dis. 2018;24(10):1835-1839. https://doi.org/10.3201/eid2410.180032

Medium

Paracoccidioides brasiliensis

- Actually several different haplotypes and even different phylogenetic species, two are most common (Adolfo Lutz, 1908, WHO, 1971). Officially *Para*. *brasiliensis* and *Para*. *lutzii*
- *Para*. *brasiliensis* and *Para*. *americana* (?? legit.) are sympatric and share similar clinical features and habitat, where they may compete for similar hosts





Variable analyzed	*P. brasiliensis* (n = 41)		*P. americana* (n = 6)		*p* value[b]
	n	% (95% CI[a])	n	% (95% CI)	
Outcome					1.0000
Cure	26	63 (49–78)	5	80 (45–100)	
Death due to PCM	4	10 (3–23)	0	0	
Complications					
Dysphonia	7	17 (6–29)	2	33 (0–71)	0.3222
Low adrenal reserve	5	16 (3–28)	3	40 (0–83)	0.0812
Cholestasis	4	10 (1–19)	0	0	1.0000
Palatal perforation	3	7 (0–15)	0	0	1.0000
Tracheostomy	2	5 (0–11)	0	0	1.0000
Microstomy	2	5 (0–11)	0	0	1.0000
Portal hypertension	2	5 (0–11)	0	0	1.0000

[a] CI–confidence interval

[b] *p* value based on Fisher's exact test

https://doi.org/10.1371/journal.pntd.0007309.t004

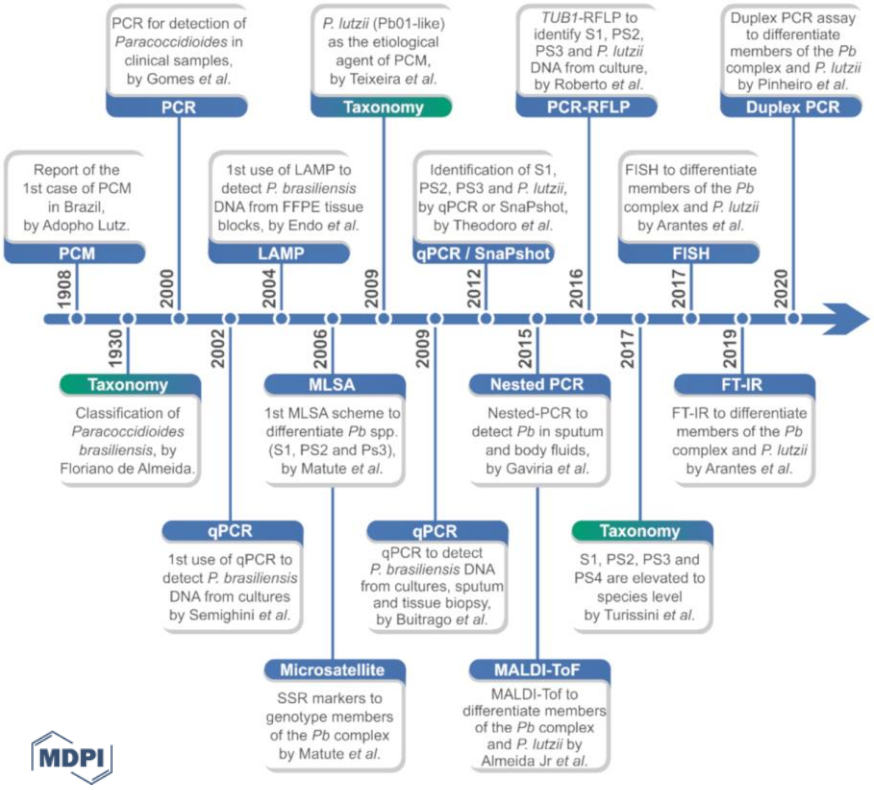
Source: De Macedo, P., Teixeira,M., Barker, B.M., et al; **Clinical features and genetic background of the sympatric species *Paracoccidioides brasiliensis* and *Paracoccidioides americana*;** *PLOS Neglected Tropical Diseases*, April 15, 2019

Paracoccidioides brasiliensis

Medium

Clinical Laboratory Diagnostics

- Major developments in the clinical laboratory identification of *Paracoccidioides* species
  - qPCR: quantitative real-time polymerase chain reaction
  - LAMP: loop-mediated isothermal amplification
  - FFPE: formalin-fixed paraffin-embedding
  - MLSA: multilocus sequence analysis
  - SSR: single sequence repeats
  - SnaPshot: single-nucleotide polymorphism (SNP) genotyping
  - MALDI-ToF: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
  - PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism
  - TUB1: tubulin alpha-1 chain
  - FISH: fluorescence in situ hybridization
  - FT-IR: Fourier-transform infrared spectroscopy



Source: Pinheiro, Breno Gonçalves, Rosane Christine Hahn, Zoilo Pires de Camargo, and Anderson Messias Rodrigues. 2020. "***Molecular Tools for Detection and Identification of Paracoccidioides Species: Current Status and Future Perspectives***" *Journal of Fungi* 6, no. 4: 293. https://doi.org/10.3390/jof6040293

Journal of Fungi

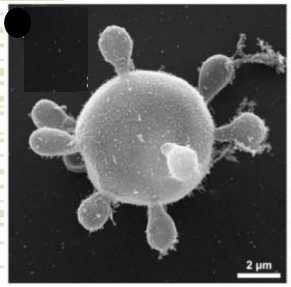
Paracoccidioides brasiliensis

Medium

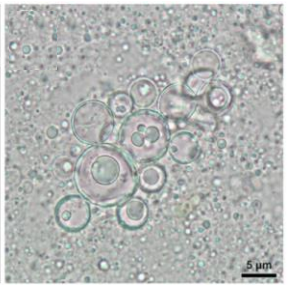
Clinical Laboratory Diagnostics

Paracoccidioides brasiliensis

Paracoccidioides lutzii



Paracoccidioides lutzii = "Pilot's wheel" (**SEM**)



+ Direct (pus) = large yeasts (5–15 µm), thick, birefringent cell wall, single or multiple buds

- SABHI
  - Chlamydoconidia
- BHI, BAP
  - Pilot's Wheel

Source: De Macedo, P., Teixeira,M., Barker, B.M., et al; **Clinical features and genetic background of the sympatric species *Paracoccidioides brasiliensis* and *Paracoccidioides americana*;** *PLOS Neglected Tropical Diseases*, April 15, 2019

Paracoccidioides brasiliensis

Medium

Clinical Laboratory Diagnostics

Paracoccidioides brasiliensis

Paracoccidioides lutzii

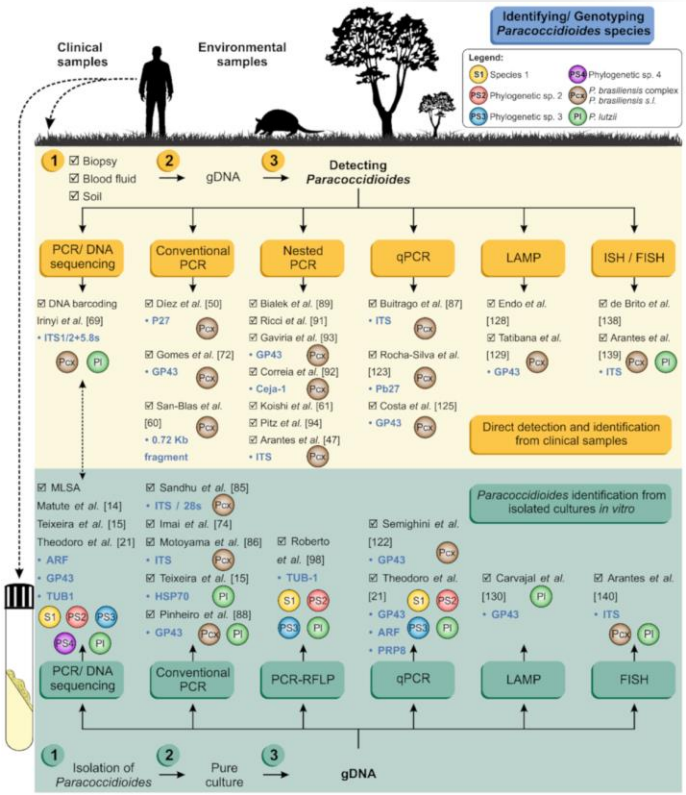
Yellow Panel: Schematic representation of spp. molecular detection/identification pipeline, directly from clinical and/or environmental samples

Green Panel: Or from gDNA extracted from cultured isolates

gDNA = Genomic DNA (Whole Genome Sequencing)

Various molecular clinical laboratory techniques;

- PCR, polymerase chain reaction
- RFLP, restriction fragment length polymorphism
- qPCR, quantitative real-time polymerase chain reaction
- FISH, fluorescent in situ hybridization
- LAMP, loop-mediated isothermal amplification



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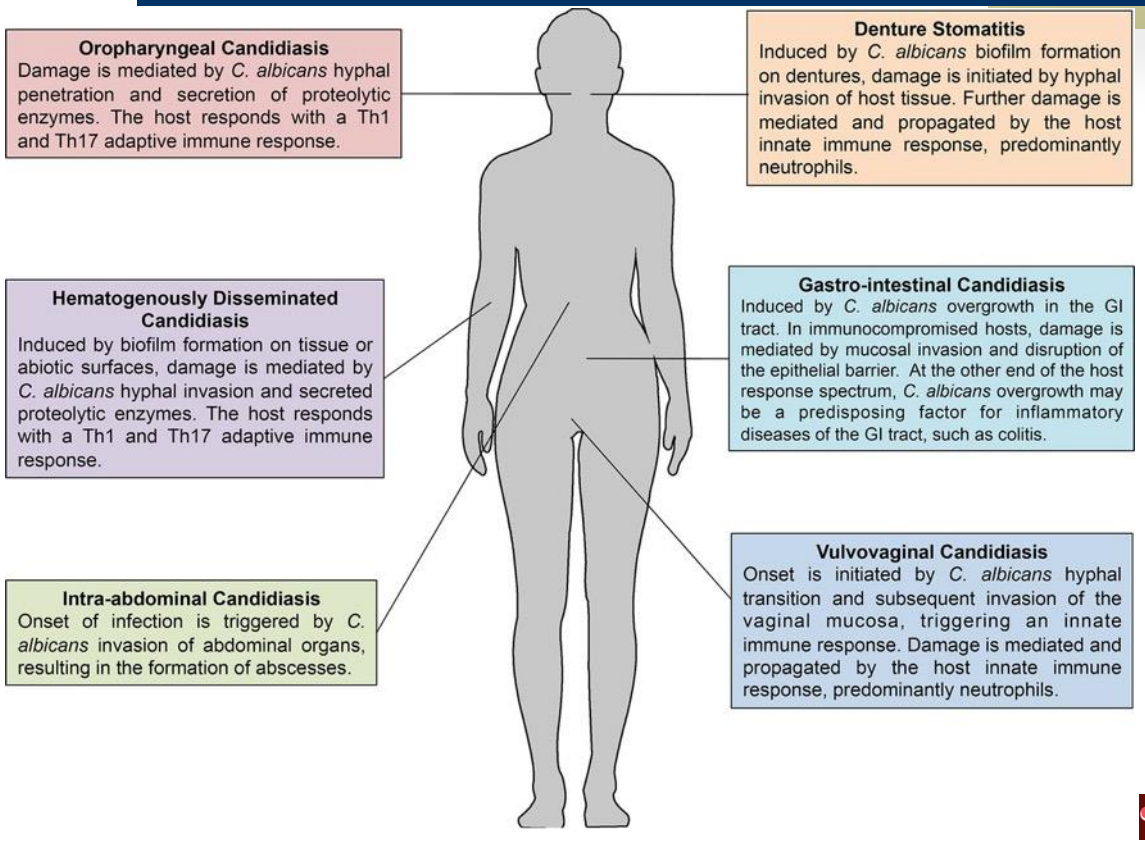
Journal of Fungi

Candidiasis

- Range of disorders
- Candidiasis = broad term
  - Cutaneous, mucosal and deep-seated organ infections
  - Can occur at any age, usually in the setting of easily identifiable risk factors for infection
- Invasive candidiasis
  - CVS
  - Deep-seated infection
    - intra-abdominal abscess
    - peritonitis
    - osteo- myelitis (with or without candidemia)

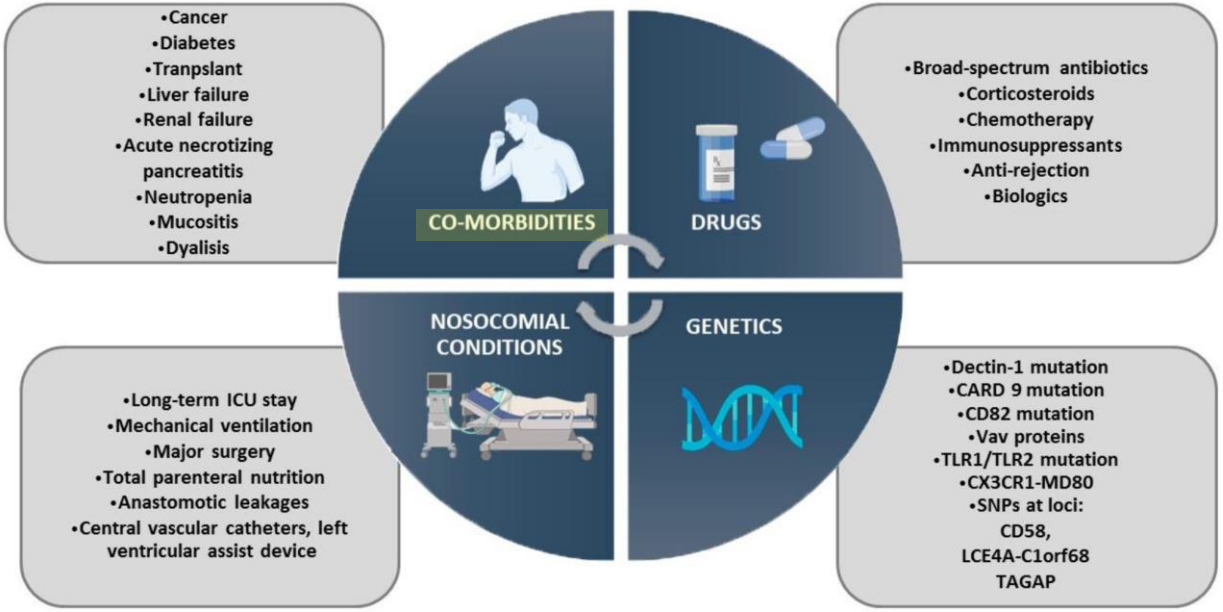


antibiotics

Source: Riera,F.O., Caeiro,J.P., Angiolini, S.C., et.al.; **Invasive Candidiasis: Update and Current Challenges in the Management of This Mycosis in South America;** *Antibiotics* **2022**, *11*(7), 877, **30 June 2022**

Candidiasis

- Leading Risk Factors;
  - 1) Hospitalization in ICUs
    - Seriously ill patients
    - mechanical supports
    - parenteral nutrition
    - difficult eradication of Biofilms
  - 2) Co-Morbidities
  - 3) Complicating pharmaceuticals
  - 4) Genetics



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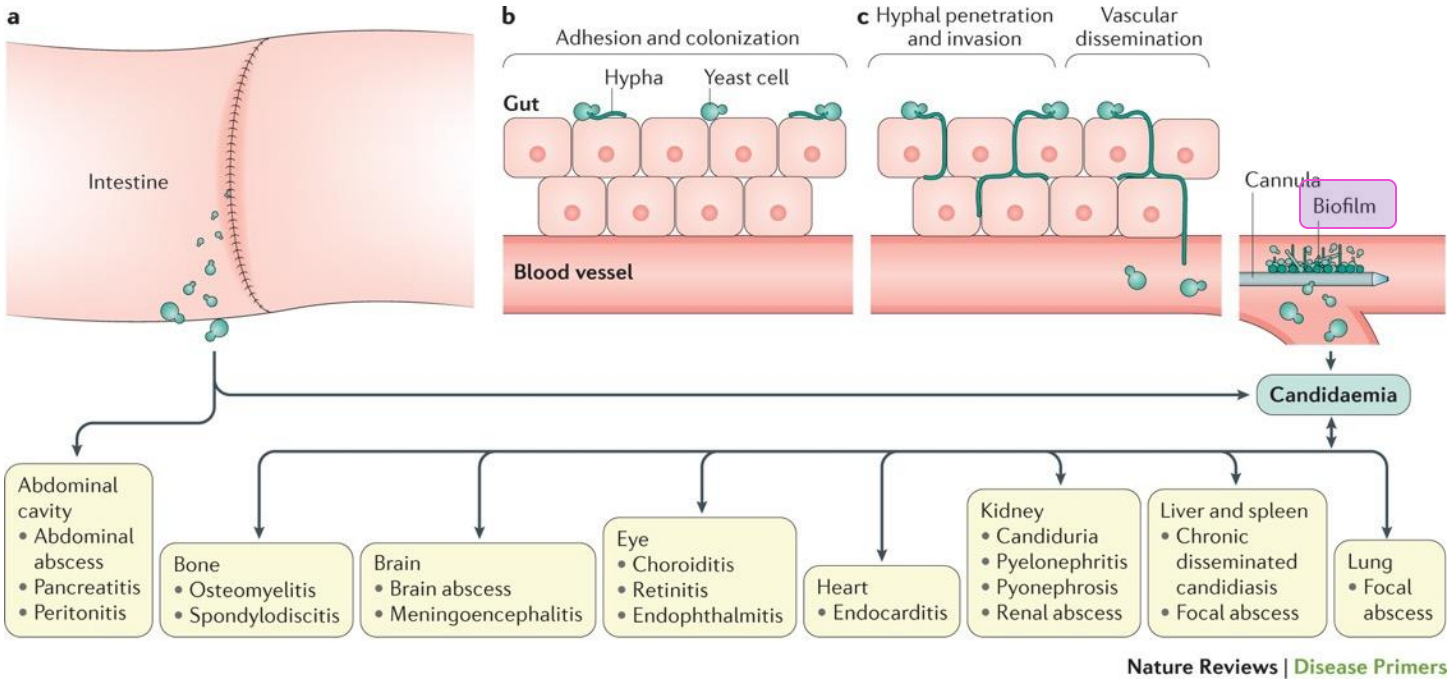
Candidiasis

- RF → Pathogen

50-70% Healthy Persons → GIT Breach → Candidemia

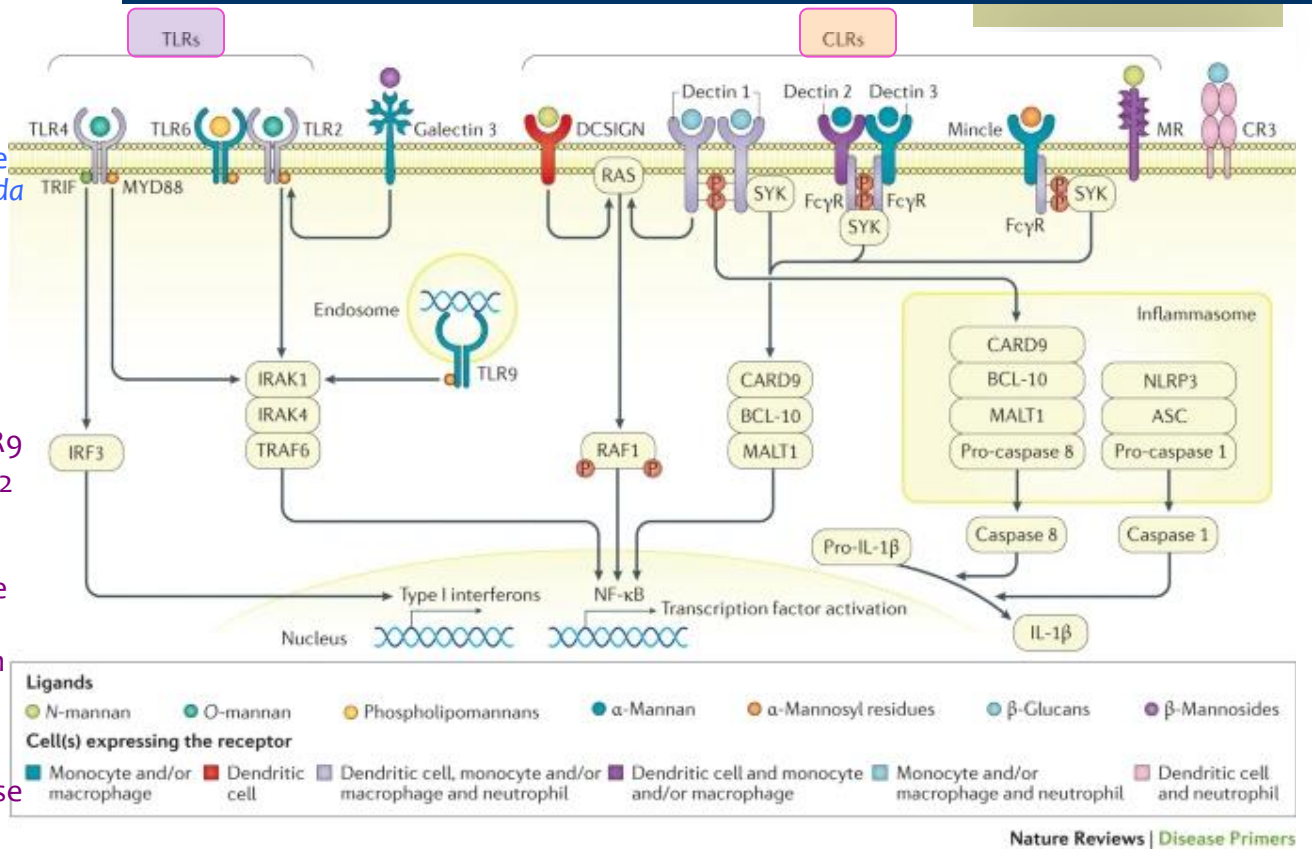


Source: Pappas,P.G., Lionakis,M.S., Arendrup, M.C., et.al.; ***Invasive Candidiasis***; Nat. Rev.; 18026, 11 May 2018

Candidiasis

- **Surface and intracellular pattern recognition receptors**
  - Different myeloid phagocytes that express them and the corresponding *Candida* spp. pathogen-associated molecular patterns (ligands)
  - Cell-surface Toll-like receptor 4
    - (TLR4)
    - intracellular TLR9
    - cell-surface TLR2
    - = forms heterodimers with cell-surface TLR6
    - = signal through the adaptor protein myeloid differentiation primary response protein MYD88

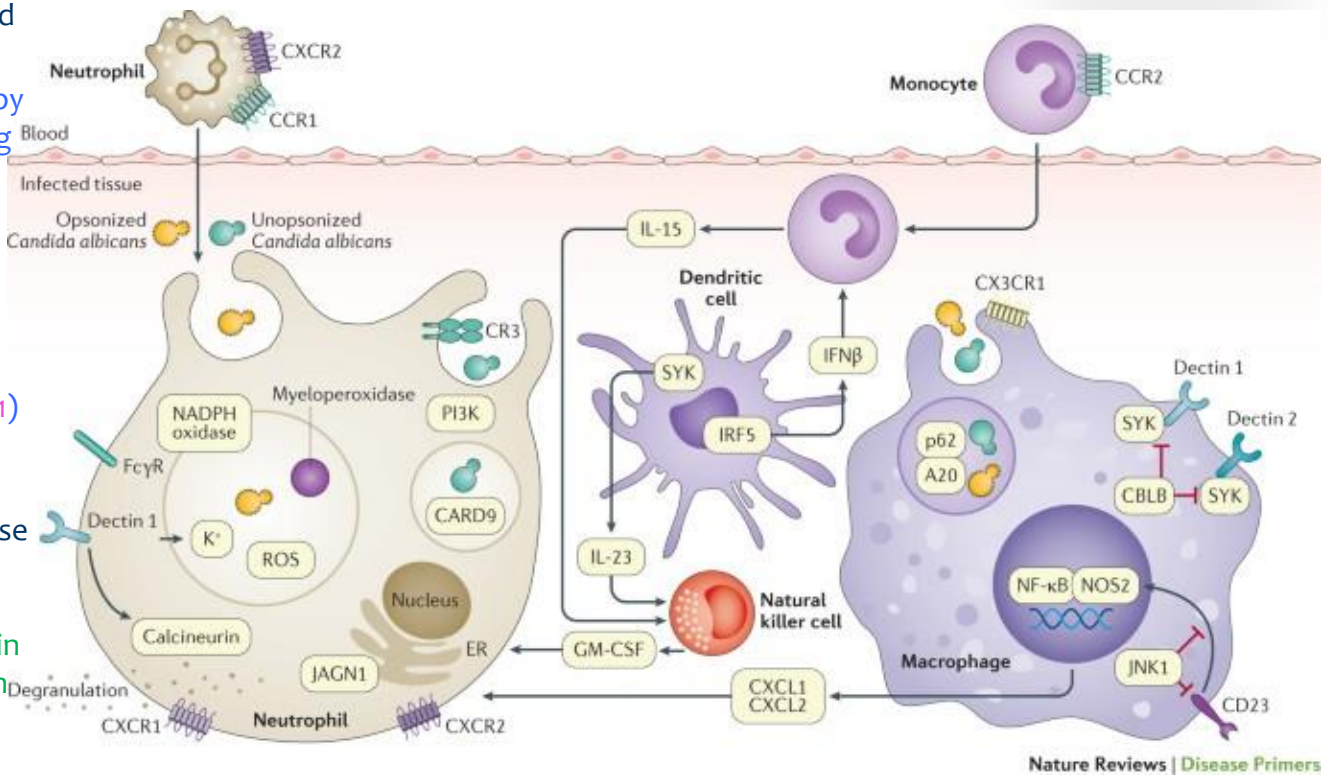
C-type lectin receptor (CLR) family of receptors recognize fungal carbohydrates



Source: Pappas,P.G., Lionakis,M.S., Arendrup, M.C., et.al.; ***Invasive Candidiasis***; Nat. Rev.; 18026, 11 May 2018

Candidiasis

- ***Tissue defense***
- Neutrophils = recruited to infected tissue
  - IL-15 produced by CCR2-expressing into tissue via CXC-chemokine receptor 2 (CXCR2) (early) and CC-chemokine receptor 1 (CCR1) (late)
- ***Killing*** = NADPH oxidase complex and dendritic cell-associated C-type lectin 1 (dectin 1)–calcineurin



Source: Pappas,P.G., Lionakis,M.S., Arendrup, M.C., et.al.; ***Invasive Candidiasis***; Nat. Rev.; 18026, 11 May 2018

Candidiasis

Clinical Laboratory Diagnostics

Diagnostic test	Specimen(s)	Advantages	Disadvantages
Fungal culture	Blood	• Enables species identification and subsequent susceptibility testing	• Slow (median detection time 2–3 days) • Sensitivity suboptimal, particularly if high volume (≥60 ml) and a fungal blood culture bottle are not employed
	Tissue and sterile body fluids	• Enables species identification and subsequent susceptibility testing	• Selective media, proper spreading of the sample and 3 days of incubation required for optimal performance
Microscopy	Cerebrospinal fluid, tissue and sterile body fluids	• Highly sensitive, particularly if using fluorescent brightener staining	• No species identification • Lower sensitivity in absence of fluorescent brightener staining
Histopathology	Tissue and sterile body fluids	• Enables evaluation of tissue invasion and inflammation	• No species identification • Lower sensitivity in absence of fluorescent brightener staining
Mannan antigen and antimannan antibody detection	Serum or plasma (EDTA) or cerebrospinal fluid	• Increased diagnostic sensitivity when combined antigen and antibody testing is performed (although in neonates (in any sample) and in cerebrospinal fluid, antigen testing suffices)	• Heavy colonization (many non-sterile body sites culture positive for *Candida* spp. and/or with heavy growth in semi-quantitative culture) could cause positivity for blood testing
β-D-glucan detection	Serum or plasma (EDTA)	• Pan-fungal marker	• No separation between *Candida* spp. and other fungi • Many sources for false positivity
PCR	Blood (EDTA)	• Rapid tests • Some commercial tests are FDA approved	• Commercial tests are expensive • May not detect all species

Critical

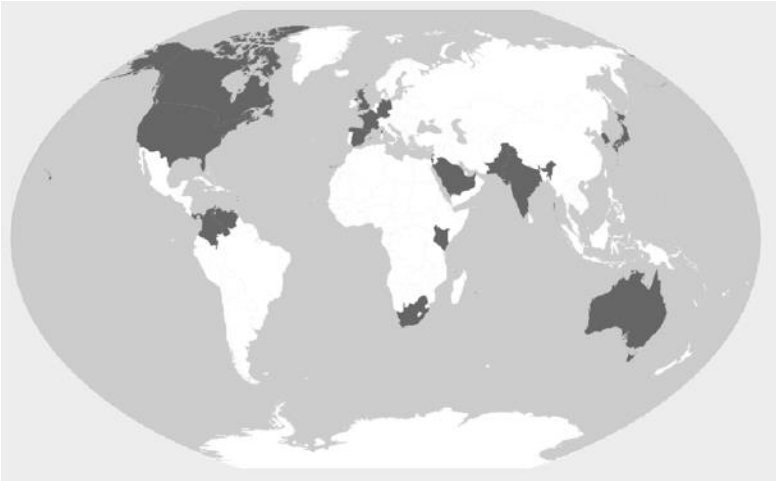
Candida auris

- ***Candida auris***, first appeared in the late 1990s
- Spread rapidly across the globe
- Killing as many as 2/3 of patients
  - Spores that travel through the CVS and "bloom" in major organs

	2016	2018	2019	2020	2021	2022
U.S. hospital cases	53	330	476	1,500	1,471	2,377

- Facilitated by its ability to **colonize skin** and other body sites, as well as its ability to **persist for weeks** on surfaces and equipment
- Divided into four distinct clades
  - Each clade, showed the ability to fight off at least one drug from the three major classes of antifungals:
    - Azoles
    - Polyenes
    - Echinocandins
  - Many resistant to two drugs, and a few samples of Clade #I were impervious to three

Countries from which ***Can. auris*** samples were taken



Credit: Amanda Montañez; Source: "Tracing the Evolutionary History and Global Expansion of *Candida auris* Using Population Genomic Analyses," by Nancy A. Chow et al., in *American Society for Microbiology*, Vo. 11; April 28, 2020

By 2020, 1,500 cases in 23 U.S. states, anti-COVID Rx made the ***Can. auris*** cases worse

Source: ***WHO fungal priority pathogens list to guide research, development and public health action***. Geneva: World Health Organization; 2022. License: CC BY-NC-SA 3.0 IGO

Critical

Candida auris

- Patients in long-term care facilities have been particularly hard hit by ***Candida auris***
- As if there weren't enough ID challenges in these settings;
  - Outbreaks, pandemics, and epidemics;
    - Scabies, severe acute respiratory syndrome (SARS), H1N1 influenza, seasonal influenza, norovirus, and multidrug-resistant organisms (MDROs) such as methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant Enterobacterales (CRE), and organisms producing extended-spectrum β-lactamases
- These patients often present with other comorbidities
  - Useful Signs & Symptoms include;
    - Fever and chills (do not improve after antimicrobial for a suspected bacterial ID)
    - CVS infection
  - > 1 in 3 patients die within a month of receiving a diagnosis of an invasive *Can. auris* infection

- Identification:
  - Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) [not all the reference databases included in MALDI-TOF devices allow for detection]
  - Real-time PCR
  - Molecular methods based on sequencing the D1-D2 region of the 28s rDNA or the Internal Transcribed Region (ITS) of rDNA also can identify *C. auris*.
  - The GenMark ePlex Blood Culture Identification Fungal Pathogen (BCID-FP) Panel and BioFire FilmArray BCID2 have been FDA approved as molecular tests for *C. auris* identification in positive blood cultures.

Source: Spaulding, L.; ***Preparing for a Candida auris Outbreak in Long-Term Care;*** Infection Control Today, June 20, 2023, (Vol. 27 No. 5) Volume 27, Issue 5

Critical

Candida auris

Current known MIS-IDENTIFICATIONS:

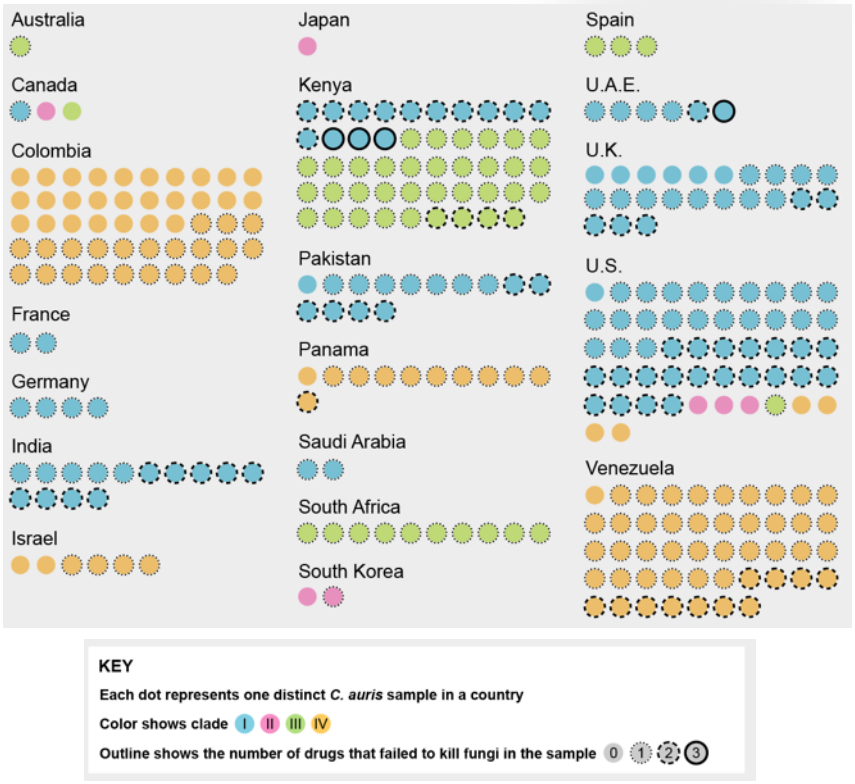
Identification Method	Organism *C. auris* can be misidentified as
Vitek 2 YST* *There have been reports of *C. auris* being misidentified as *Candida lusitaniae* and *Candida famata* on VITEK 2. A confirmatory test such as cornmeal agar may be warranted for these species.	*Candida haemulonii* *Candida duobushaemulonii*
API 20C	*Rhodotorula glutinis* (characteristic red color not present) *Candida sake*
API ID 32C	*Candida intermedia* *Candida sake* *Saccharomyces kluyveri*, *cervisiae*
BD Phoenix yeast identification system	*Candida haemulonii* *Candida catenulata*
MicroScan **On cornmeal agar, *C. guilliermondii*, *C. lusitaniae*, and *C. parapsilosis* generally make pseudohyphae and *C. auris* does not make hyphae or pseudohyphae. If hyphae or pseudohyphae are not present on cornmeal agar, any *C. guilliermondii*, *C. lusitaniae*, and *C. parapsilosis* isolates identified on MicroScan or any *C. parapsilosis* isolates identified on RapID Yeast Plus should be submitted for further identification	*Candida famata* *Candida guilliermondii*** *Candida lusitaniae*** *Candida parapsilosis***
RapID Yeast Plus	*Candida parapsilosis***

Source: CDC

Critical

Candida auris

- ***Candida auris*** from WHO study
- Lives quiescently in GIT then surges out into their CVS or onto mucous membranes when their immune system shifted out of balance
- Early 21st Century, gained ability to:
  - directly pass from person to person
  - live on metal, plastic, and the rough surfaces of fabric and paper
    - Reuse surgical masks/gowns (COVID)

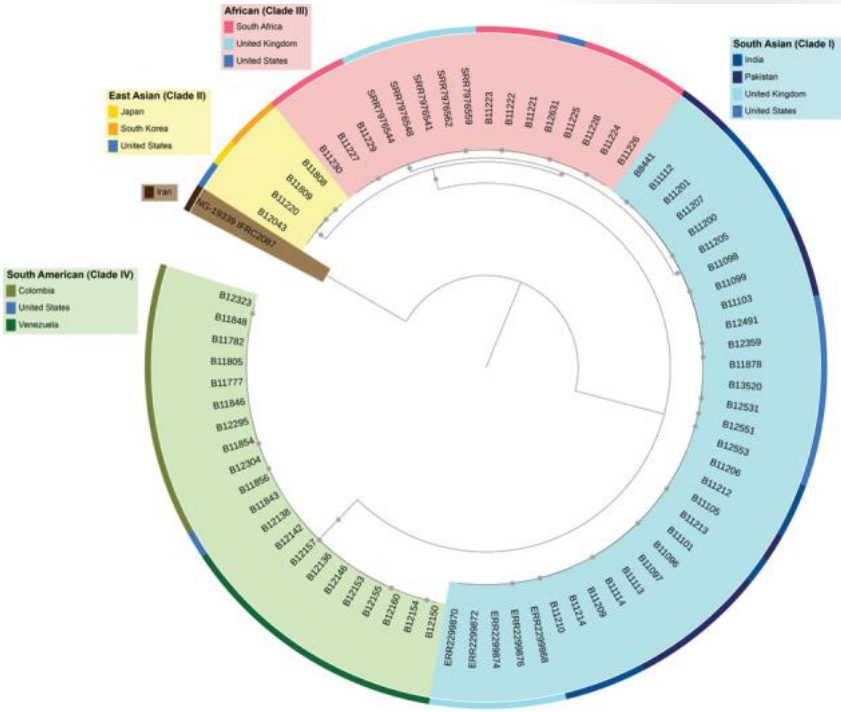


Source: "***Deadly Kingdom***" ; Scientific American 324, 6, 26-35 (June 2021); doi:10.1038/scientificamerican0621-26

Critical

Candida auris

- Major clades of ***Candida auris***
- ***Clade #1 – South Asian***
  - *India, Pakistan, UK, U.S.*
- ***Clade #2 – East Asian***
  - *Japan, South Korea, U.S.*
- ***Clade #3 – African***
  - *South Africa, UK, U.S.*
- ***Clade #4 – South American***
  - *Coloumbia, U.S., Venezuela*
- ***Clade #5 – Iran*** (*Tentative - 2018*)
  - Distinct via: >200,000 single-nucleotide polymorphisms, in a 14 y.o. Girl (Otomycosis) in Iran who had never traveled outside the country
- Maximum-likelihood phylogenetic tree shows isolates from ***Can. auris*** cases from 10 countries. Circles at nodes indicate separations with a bootstrap value >99%

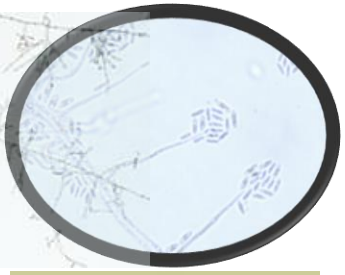


Source: Chow,N.A., de Groot, T., Badali, H., et al; ***Potential Fifth Clade of Candida auris, Iran, 2018;*** Emerg Infect Dis. 2019 Sep; 25(9): 1780–1781

EMERGING INFECTIOUS DISEASES

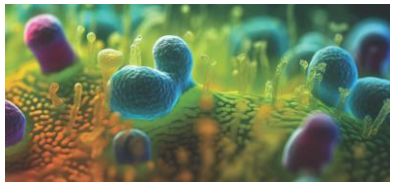
Critical

Rx for Can. auris (Tent.)



- Current treatment guidelines
- Echinocandins are the DOC generally
  - Res. = ↑ 3X

Echinocandin Drug	Adult dosing	Pediatric dosing
Anidulafungin	loading dose 200 mg IV, then 100 mg IV daily	not approved for use in children
Caspofungin	loading dose 70 mg IV, then 50 mg IV daily	loading dose 70mg/m^2/day IV, then 50mg/m^2/day IV (based on body surface area)
Micafungin	100 mg IV daily	2mg/kg/day IV with option to increase to 4mg/kg/day IV in children at least 40 kg
Adults and Children ≥ 2 M.O.		
Neonates, infants < 2 M.O.	**Neonatal dosing**	
Caspofungin	25 mg/m^2/day IV (based on body surface area)	
Micafungin	10mg/kg/day IV	

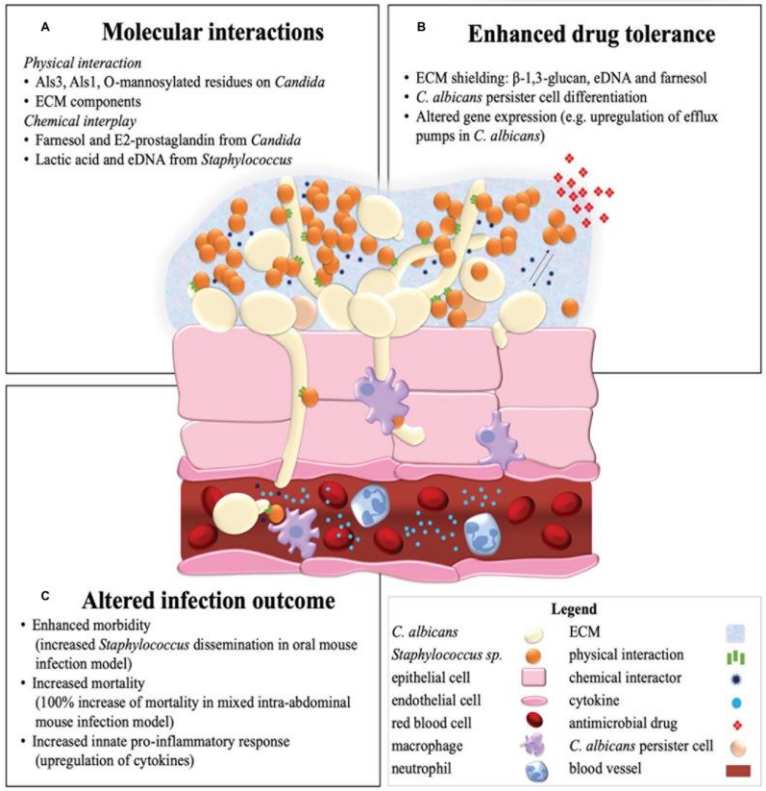


Source: **Treatment and Management of Infections and Colonization**; CDC, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED); 7/22/21

Critical

Candida albicans

- Summary illustration of a mixed ***Can. albicans*** and ***Staphylococcus*** species biofilm in a host environment
- (A) Invasive endothelial infection with an enhanced antimicrobial drug tolerance
- (B) Enhanced Drug Tolerance
- (C) Altered Infection outcome
- Coexists with *Staphylococcus epidermidis* & *Sth. aureus* but can cause enhanced infections with them. Now shown to create complex infective biofilms
- Secreted effectors such as quorum sensing (QS) molecules and small secreted metabolites are involved in communication, drug tolerance
- Representative infections;
  - **Thrush** — O.T. infection. Can affect any moist surface around the lips, inside the cheeks, and on the tongue and palate
  - **Esophagitis** — Candida infections of the mouth can spread to the esophagus, causing esophagitis



Source: Centers for Disease Control and Prevention; ***Mycotic Disease Branch***

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Critical

Candida albicans

- Representative infections;
  - **Cutaneous Candidiasis** — Common skin infections, in areas of skin that receive little ventilation and are unusually moist;
    - Diaper area
    - Hands of people who routinely wear rubber gloves
    - Rim of skin at the base of the fingernail, especially for hands that are exposed to moisture
    - Groin and in the crease of the buttocks
    - Skin folds under large breasts.
  - **Vaginal yeast infections** — Not usually transmitted sexually, but can be
    - 75% of all women are likely (lifetime) to have at least one vaginal Candida infection, ~45% have 2 or more
    - Pregnant or Diabetic
    - Antimicrobials, birth control pills, frequent douching can promote
  - **Deep candidiasis** (Ex. Candida sepsis) — CVS or Systemic
    - Newborns with very low birth weights
    - Anyone with severely weakened immune system (Ex. anticancer drugs)
    - Entrance to CVS through;
      - skin catheters, tracheostomy sites, ventilation tubing, or surgical wounds
      - IV drug abuse, severe burns or wounds caused by trauma

Source: Carolus,H., vanDyck,K., and vanDijck; *Candida albicans* and *Staphylococcus* Species: A Threatening Twosome; Front. Microbiol., 18 September 2019 Sec. Fungi and Their Interactions ; https://doi.org/10.3389/fmicb.2019.02162

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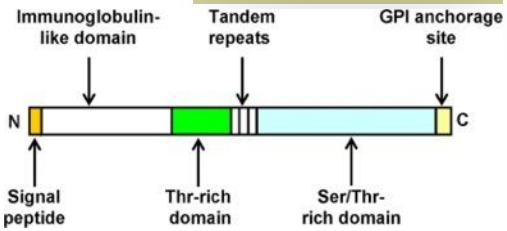
Critical

Candida albicans

- **Virulence Factors;**
  - **Polymorphism**
    - Yeast, pseudohyphae and hyphae
    - Hyphae is more important for infection
  - **Adhesins (Als 3 Protein)**
    - Sets of glycosylphatidylinositol (GPH)- linked cell surface glycoproteins that allow it to adhere to the surfaces of microorganisms
    - Helps with biofilm formation also
  - **Invacins (Als 3 Protein)**
    - Helps with the invasion of ***Can. albicans*** into host epithelial and endothelial cells.
    - Ssa1 codes for heat shock protein
    - Induces host cells to engulf the fungal pathogens
    - Invasion by the active penetration of ***Can. albicans*** into host cells by involving hyphae
  - **Biofilm Formation**
    - Yeast cells, adherence & surface development of hyphae cells in the upper part of biofilm, leads to a more resistant mature biofilm dispersion of yeast cell
    - Bcr1, Tec1 and Efg1 function as important transcriptional factors

Schematic diagram of the structure of **Als3 Protein**



Source: Liu, Y., and Filler, S.G.; **Candida albicans Als3, a multifunctional adhesin and invasion;** Eukaryot Cell. 2011 Feb; 10(2): 168–173. doi: 10.1128/EC.00279-10

Source: Carolus,H., vanDyck,K., and vanDijck; *Candida albicans* and *Staphylococcus* Species: A Threatening Twosome; Front. Microbiol., 18 September 2019 Sec. Fungi and Their Interactions ; https://doi.org/10.3389/fmicb.2019.02162

Critical

Candida albicans

- **Virulence Factors**;
  - **Secreted hydrolases**
    - 3 main classes of hydrolases: proteases, phospholipases and lipases
    - Helps in active penetration into host cells
    - Helps in uptake of extracellular nutrients from the environment
    - 10 proteases (Sap 1-10), 4 major classes (A, B, C and D) of phospholipases and lipases consist of 10 members (LIP 1-10)
  - **Metabolic Adaption**
    - In the process of infection, it undergoes metabolic adoption such as their glycolysis, gluconeogenesis and starvation responses.
    - Example: quickly switch from its glycolysis to starvation responses with the activation of glyoxylate cycle
    - Due to this, it can infect almost any organ through the blood stream.
- NOTE: ***Candida*** = **314 species** accepted with the type species *Candida vulgaris* (Berkhout, et.al.)

Source: Carolus,H., vanDyck,K., and vanDijck; *Candida albicans* and *Staphylococcus* Species: A Threatening Twosome; Front. Microbiol., 18 September 2019 Sec. Fungi and Their Interactions ; https://doi.org/10.3389/fmicb.2019.02162

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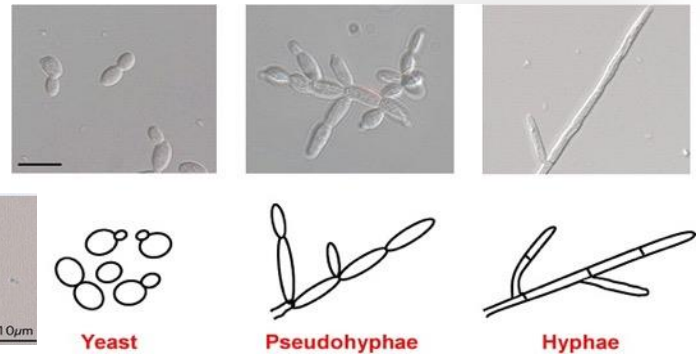
Candida albicans

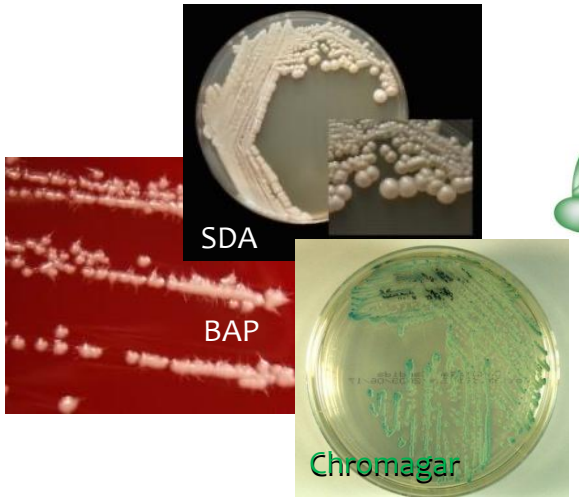
Critical

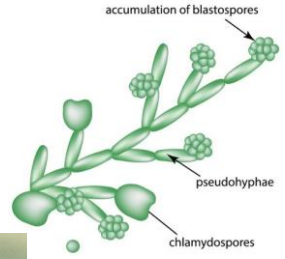
Clinical Laboratory Diagnostics

Candida albicans

- Small, oval, measuring 2-4 µm in diameter (10% KOH)
- **Yeast** form, unicellular, reproduce by budding.
- Single budding of the cells may be seen.
- Both yeast and **pseudo-hyphae** are **gram-positive**
- Germ Tube (+) @ 2hr. Or <, human serum, 37°C
- Encapsulated and diploid, also form true hyphae.
- Polymorphic fungus (yeast and pseudohyphal form)
- Can form biofilms
- Normal condition: Yeast
- Special condition (pH, Temperature): Pseudohyphae
- 80-90% of cell wall is carbohydrate
- **Culture**;
  - **SDA** = Creamy, pasty colonies, smooth after 24-48 hours at 25-37° C
  - "Yeasty" smell
  - **BAP** = White creamy colored
  - Foot-like extensions from the margin
  - **Chromagar** = Green
  - Chlamydospores @ 25°C, Corn Meal Agar / Rice Agar







Source: Aryal,S.; ***Candida albicans*- *An Overview***; Microbe Notes, 5/15/22

Nakaseomyces glabrata

High

Species Complex

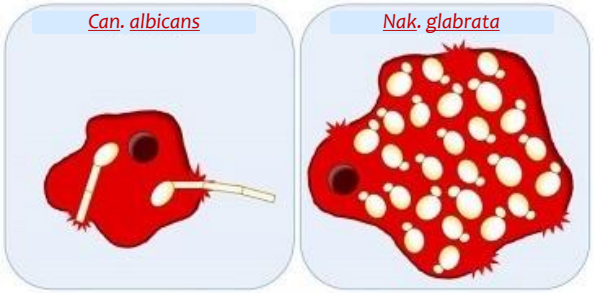
Candida glabrata

Nakaseomyces glabrata

Nak. nivariensis

Nak. bracarensis

Nakaseomyces = Teleomorph, this is now the official genus-level name, but "Candida" is the recommended "reporting" name.



Can. albicans can "escape" from a Mφ whereas Nak. glabrata just outlasts it

- *Candida* infections remain by far the most frequent cause of **IFI** (**I**nvasive **F**ungal **I**nfections)
  - = 43%–75% of **IFI** (Est. = 36 to 290 / million pop.)
  - Now #2 behind ***Can. albicans*** (distant clade, (-) Pseudohyphae, really closer to ***Saccharomyces*** *spp.*)
- 1st ID (1917) as a ***Cryptococcus***, by H. W. Anderson
- ***Torulopsis glabrata*** (1938), Lodder and De Vries ((-) Pseudohyphae) , but a commensal)
- \+ Mucosal and invasive candidiasis (1988), Odds, et.al.
- The main concern with ***Nak. glabrata*** infection is life-threatening invasive forms;
  - Candidemia = CVS dissemination, = → liver → spleen → kidney
    - age (>60 years), an underlying solid tumor, a recent abdomino-pelvic surgery, previous antimicrobial therapy
      - third generation cephalosporins, tazocilline, vancomycin or prior treatment with fluconazole or echinocandins
  - Intra-abdominal (most common)

Source: ***Larone's Medically Important Fungi: A Guide to Identification***, Westblade, L.F., Burd, E.M., Lockhart,S.R., and Procop,G.W.; ASM Press, Washington,D.C., 2023

CDC

Nakaseomyces glabrata

High

Clinical Laboratory Diagnostics

Species Complex

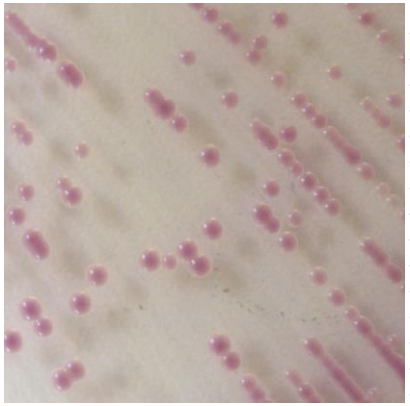
Candida glabrata

Nakaseomyces glabrata *sensu stricto*

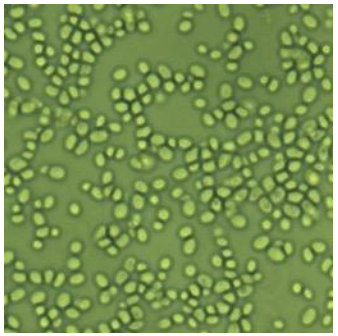
Nak. nivariensis

Nak. Bracarensis

Nakaseomyces = Teleomorph



CHROmagar



Culture

- On SDA incubated@ 37°C; ***Nak. glabrata***, ***Nak. nivariensis*** and ***Nak. bracarensis*** grow in the form of white or creamy butyrous colonies
- budding; cells are small (3–4.5 µm), subglobose to ovoidal
- ChromAgar medium, ***Nak. glabrata*** can usually be differentiated by producing pink colonies while the two others remain white

Molecular Typing

- AFLP, RAPD and PCR finger printing

Feature	*Nak. glabrata* ATCC 2001 = CBS 138	*Nak. nivariensis* CBS 9983 = CECT 11998	*Nak. bracarensis* CBS 10154 = CECT 12000
Growth			
Surface growth(37°C)	–	–	–
SDA cycloheximide (25°C)	–	–	+
SDA (37°C)	+	+	+
YPD (30°C)	+	+	+
YPD (45°C)	–	–	–
Turbidity YPD (37°C) (Yeast Extract Potato Dextrose)	+	–	–
Assimilation			
Dextrose	–	–	–
Maltose	–	–	–
Sucrose	–	–	–
Lactose	–	–	–
Galactose	–	–	–
Melibiose	–	–	–
Cellobiose	–	–	–
Inositol	–	–	–
Xylose	–	–	–
Raffinose	–	–	–
Trehalose	+	+	+
Dulcitol	–	–	–
KNO3	–	–	–
2-Keto-gluconate	–	–	–
Glycerol	+	+	+
Fermentation			
Dextrose	+	+	+
Maltose	–	–	–
Sucrose	–	–	–
Lactose	–	–	–
Galactose	–	–	–
Trehalose	+	+	+
Cellobiose	–	–	–
Other characteristics			
Germ tubes	–	–	–
Urease (25°C)	–	–	–
Ascospores	–	–	–
Chlamydospores	–	–	–
Esculin hydrolysis	–	–	–
Pseudohyphae	–	–	–

Source: Angoulvant,A., Guitard,J., and Hennequin, C.; **Old and new pathogenic *Nakaseomyces* species: epidemiology, biology identification, pathogenicity and antifungal resistance;** *FEM S Yeast Research*, Volume 16, Issue 2, M arch 2016, fov114

Candida tropicalis

- *Candida tropicalis* has been identified as the most prevalent pathogenic yeast species of the Candida-non-albicans (CNA) group. *Can. albicans* is still a leader in human infections, but *Can. tropicalis* is rising FAST
  - = Unexplained resistance to fluconazole
  - Unclear mechanism of pathogenicity
  - Odd consequent immune response
- CVS infections caused by *Candida* species
  - Resultant annual high rate of mortality worldwide
  - Significant hospital costs of $1.4 billion in the US each year
  - Most common causes = *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Nakaseomyces glabrata* (*Can. glabrata*), *Pichia kudriavzveii* (*Can. krusei*)
  - *Nakaseomyces glabrata* (*Can. glabrata*), *Pichia kudriavzveii* (*Can. krusei*) = higher minimum inhibitory concentration (MIC) values toward azoles
    - Azole resistance mechanisms in *Can. albicans*, *Can. parapsilosis*, and *Can. tropicalis* is mediated mainly by the occurrence of specific amino acid substitutions in **ERG11**, resulting in reduced affinity of azoles to the drug target, in addition to overexpression of efflux pumps
  - *Nakaseomyces glabrata* (*Can. glabrata*), = rapidly acquires resistance to echinocandins
  - CVS isolates of *Candida tropicalis*, *Candida parapsilosis* have been demonstrating RESISTENCE to Fluconazole
  - **MDR strains** of *Nakaseomyces glabrata* (*Can. glabrata*), and *Can. auris* are increasing worlwide

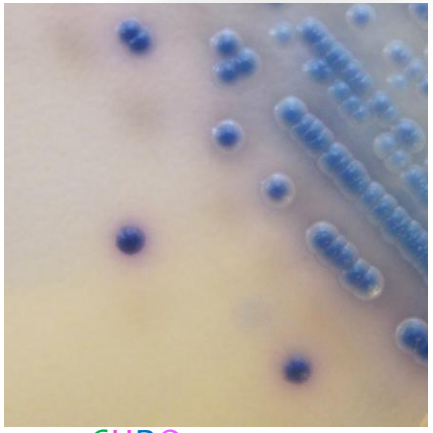
Source: Kothavade,R.L., , Kura,M.M.,Valand,A.G., et.al.; ***Candida tropicalis: its prevalence, pathogenicity and increasing resistance to fluconazole*;** Med Microbiol. 2010 Aug;59(Pt 8):873-880, doi: 10.1099/jmm.0.013227-0.Epub 2010 Apr 22.

Candida tropicalis

High

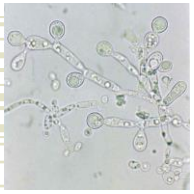
Clinical Laboratory Diagnostics

Name of species.	Microscopic characteristic			Growth on SDA	Growth on rice meal agar			Carbohydrate fermentation test.					CHROM Agar test
	Morphology	Gram's stain	Germ tube test		Presence of blasto-conidia	Presence of pseudo-hyphe	Chlymydo spores	Glu	Mal	Suc	Lac	Gal	According to color
C. albicans	Spherical to budding	+	+	+	+	+	+	+	+	–	–	+	Light-gree n
C. tropicals	Spherical to budding	+	–	+	+	+	–	+	+	±	-	+	Dark-blue
C. krusei *Pichia kudriavzveii*	Small to ovoid	+	–	+	+	+	–	+	–	–	–	–	Pale-pink
C. glabrata *Nakaseomyces glabrata*	Ovoid to bud	+	–	+	+	–	–	+	–	–	–	–	White-pink



CHROmagar

- *Culture* (SDA, PDA, CMA, CHROMagar, and AFST (Antifungal Sensitivity Testing Agar) are common)
  - *Can. tropicais* colonies on Sabouraud Dextrose Agar (SDA) are white to cream, with a creamy texture and smooth appearance, and may have slightly wrinkled edges. ∴ indistinguishable from other *Candida* species.
  - Common antifungals tested with AFST;
    - Clotrimazole, Econazole, Miconazole, Terbinafine, Fluconazole, Ketoconazole, Itraconazole, Voriconazole, Posaconazole, Ravuconazole, Amphotericin-B, 5-Fluorocytosine



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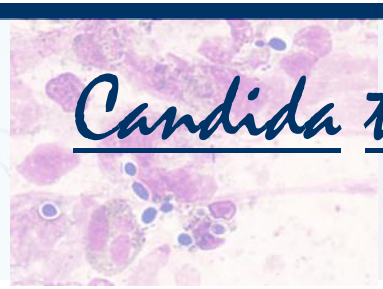
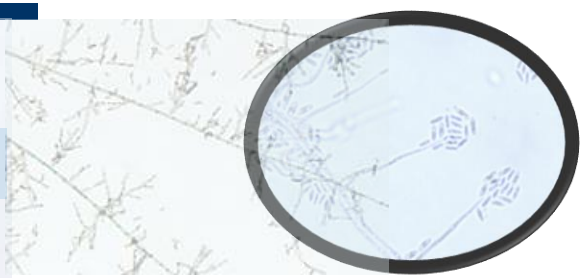
•**Source:** Zuza-Alves,D.L., Silva-Rocha,W.P., Chaves, G.; An Update on Candida tropicalis Based on Basic and Clinical Approaches; October 2017, Frontiers in Microbiology 8

Candida tropicalis

High





Clinical Laboratory Diagnostics

	Method	Principle	Advantages	Disadvantages
Classical methodology	Auxanogram and zymogram	Assimilation and fermentation of several different carbon and nitrogen souces	Easy execution and low cost	Laborious and time-consuming, subjectivity of interpretation
	Microculture on cornmeal agar containing Tween 80	Yeasts incubation on culture medium with Tween 80 and low oxygen tension esporulation and filamentation		
	Urease test	Urea hydrolysis alkalinizes the medium, causing the pH indicator to change. The medium goes from yellow to pink, indicating positivity		
Chromogenic media	Chromagar *Candida*®, *Candida* ID2®, CandiSelect4®, *Candida* Brilliance®	Different substrates react with specific enzymes of the main *Candida* species and induce the formation of colonies with different colors for tpresumptive identification	Rapid screening of different species and checking the purity of *Candida* colonies, detects mixed infections; high sensitivity and specificity	Presumptive identification for only five species of the *Candida* genus
Semi-automated methods	API 20C AUX, API ID 32C system	Galleries with different carbon sources, where growth and assimilation is observed by turbidity in the respective well	Good reproducibility and easy execution	May not be completely accurate on some cases and may lead to an incomplete identification, needing supplementary tests or even give a wrong identification for some species; higher cost. Not all the rare *Candida* species are included in the galleries
	CandiFast® system	The identification well contains cycloheximide, besides seven carbohydrates, where fermentation is analyzed after acidification and alteration of media colors due to the presence of a pH indicator	Used for identification and antifungal susceptibility testing	
	AuxaColor™ Kit	Assimilation of 13 sugars, besides the enzymatic detection of N-acetyl-galactosaminidase, phenoloxidase and L-proline arilamidase	Good reproducibility and easy execution	
Automated methods	Vitek2® System	Fluorometric and colorimetric methods for microorganism's identification and analysis in a software which contains a database with 52 yeast species	Rapid results, requires minimal preparation of reagents	
	BD Phoenix™	Polystyrene strips contain three fluorescent control wells (a negative and two positives) with 47 wells containing lyophilized substrates		

Can. *tropicalis* = Dk. Blue colonies

frontiers in Microbiology

•**Source:** Zuza-Alves,D.L., Silva-Rocha,W.P., Chaves, G.; An Update on Candida tropicalis Based on Basic and Clinical Approaches; October 2017, Frontiers in Microbiology 8

High

Species Complex

Candida parapsilosis

- ***Candida metapsilosis*, *Can. orthopsilosis*, *Can. parapsilosis*** *sensu stricto* [all VERY difficult to distinguish phenotypically]. ***Lodderomyces elongisporus*,** closely related can also be confused.
- *Candida parapsilosis* *sensu stricto* is a common fungal pathogen of low birth weight infants
  - Together with the less frequently clinically encountered pathogens *Can. orthopsilosis* and *Can. metapsilosis* they compose the *Can. parapsilosis* *sensu lato* species complex
  - Typically these organisms do not damage individuals with a competent immune system
- *Candida parapsilosis* has three genes called ***SAPP1*, *SAPP2*,** and ***SAPP3*** for secreted aspartic protease 1 (Sapp1p), secreted aspartic protease 2 (Sapp2p), and secreted aspartic protease 3 (Sapp3p), which together are known as ***candiparapsins***
- The major virulence factors of *Can. parapsilosis* are the secreted enzyme, aspartic proteases (SAPPs), which help the pathogen to disseminate, acquire nutrients, and *dysregulate the mechanisms of innate immunity* of the host
- Catheter-Related Bloodstream Infection (CRBSI) is an important healthcare-associated infection caused by various nosocomial pathogens (***prominent Biofilm (pseudohyphae) producer***)
- *Candida parapsilosis* has emerged as quite significant over the last two decades
  - Demography
  - Pre-maturity
  - Comorbidities
    - Diabetes mellitus
    - Hypertension
    - CVS disorders
    - Neuropathy
    - Respiratory diseases
    - Renal dysfunction
    - Hematological and solid organ malignancies, intestinal dysfunction
    - ICU admission
    - Mechanical ventilation (MV)
    - Prior antibiotic and/or antifungal therapy
    - Others

Source: Yamin,D.H., Husin, A., Harun, A.; **Risk Factors of *Candida parapsilosis* Catheter-Related Bloodstream Infection**; Front. Public Health, 12 August 2021

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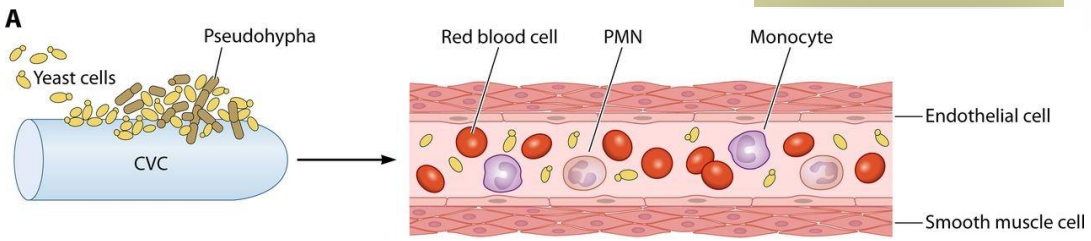
Candida parapsilosis

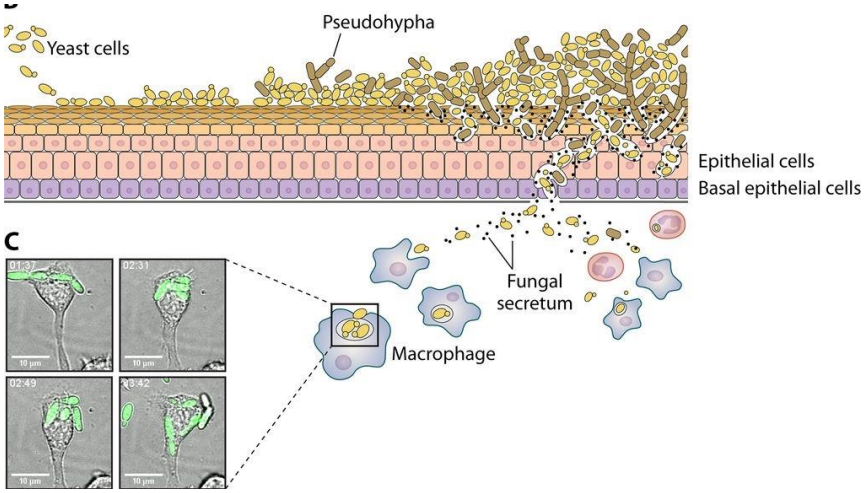
High

Candida parapsilosis

- Central venous catheter (CVC) colonization by *Can. parapsilosis* as the source of infection
- Implantation of the contaminated device results in systemic dissemination



- Colonization and invasion of host epithelial surfaces
  - various virulence factors, including morphology transition and the release of fungal secretions such as hydrolytic enzymes
- After phagocytosis, fungal cells not only survive but also may induce exocytosis or replicate within host cells



Source: Toth,R., Mora-Montes, H., Gabaldon, T.; ***Candida parapsilosis: from Genes to the Bedside***; Clin.Micro. Rev., 27 February 2019

AMERICAN SOCIETY FOR MICROBIOLOGY

- Ranking = as a cause of Invasive Candidiasis
- *Can. albicans* = #1
- *Can. parapsilosis* = often #2
- MUCH less common here

Region	% *C. parapsilosis* incidence (ranking)	% *C. albicans* incidence (ranking)	Yr(s)	No. of hospitals included
Europe				
South region				
Spain	24.9 (2nd)	45.3 (1st)	2010–2011	29 hospitals, nationwide
Italy	22 (4th)	73.4 (1st)	Undefined	3 hospitals in southern Italy
	14.8 (3rd, Lombardy)	52.1 (1st, Lombardy)	2009	34 centers, nationwide
	23.5 (2nd, other areas)	45.2 (1st, other areas)		
	20 (2nd)	59 (1st)	2012–2013	39 hospitals, northern Italy
Portugal	23 (2nd)	40.4 (1st)	2011–2012	10 hospitals, nationwide
Greece	22.7 (2nd)	45.4 (1st)	2005–2009	PICU only, nationwide
Serbia	46	46	2014–2015	5 adult ICUs, nationwide
Middle/north regions				
Finland	5 (3rd)	67	2004–2007	5 regions, nationwide
Austria	8.7 (3rd)	52.2 (1st)	2007–2008	9 centers
France	7.5 (3rd)	57 (1st)	2005–2006	180 ICUs, nationwide
United Kingdom	10.3 (3rd)	52 (1st)	2008	3 centers in Scotland, 2 centers in Wales
Switzerland	5.4 (4th)	61.9 (1st)	2004–2009	17 hospitals
Denmark	3.7 (3rd in females, 5th in males)	57.1 (1st)	2004–2009	6 hospitals
Norway	4.3 (4th)	67.7 (1st)	2004–2012	National surveillance study
Sweden	9 (3rd)	61 (1st)	2005–2006	Undefined, nationwide
Iceland	5 (5th)	56 (1st)	2000–2011	14 hospitals

Nakaseomyces glabrata is often more common here

Region	% *C. parapsilosis* incidence (ranking)	% *C. albicans* incidence (ranking)	Yr(s)	No. of hospitals included
America				
South America				
Continental study	26.5 (all episodes)	37.6 (all episodes)	2008–2010	21 hospitals from 7 countries
Argentina	23.9 (2nd)	42.5 (1st)		
Brazil	25.8 (2nd)	40.5 (1st)		
Chile	28.9 (2nd)	42.1 (1st)		
Colombia	**38.5 (1st)**	36.7 (2nd)	2008–2010	21 hospitals from 7 countries
Ecuador	30.4 (2nd)	52.2 (1st)		
Honduras	14.1 (4th)	27.4 (1st)		
Venezuela	**39 (1st)**	26.8 (2nd)		
Peru	25.3 (2nd)	27.8 (1st)	2013–2015	3 hospitals, Lima-Callao
	28.1 **(1st)**	39.9 (1st)	2009–2011	9 hospitals, Lima
Argentina	22 (2nd)	44 (1st)	2010–2012	5 institutions
Brazil	24.1 (2nd)	34.3 (1st)	2007–2010	16 hospitals, 5 regions, nationwide
North America				
Continental study	12.2 (3rd)	49.5 (1st)	2004–2008	23 centers in USA, 2 in Canada
USA	**17 (3rd)**	38 (1st)	2008–2011	17 hospitals (Baltimore, MD), 24 hospitals (Atlanta, GA)
	17.4 (2nd)	50.7 (1st)	1998–2006	52 hospitals, nationwide
Canada	21 (2nd)	59 (1st)	2003–2013	Nationwide NICU surveillance
Asia				
Continental study	12.1 (4th)	41.3 (1st)	2010–2011	25 hospitals across Asia
Japan	23.3 (2nd)	39.5 (1st)	2003–2014	10 university hospitals, nationwide
China	20.0 (2nd)	44.9 (1st)	2009–2014	65 general hospitals from 27 provinces
India	10.9 (3rd)	20.9 (2nd)	2011–2012	27 ICUs, nationwide
Oceania				
Australia	16.5 (3rd)	44.4 (1st)	2014–2015	Nationwide surveillance
Africa				
South Africa	35 (public hospitals) (2nd), >50 (private hospitals) **(1st)**	46 (1st)	2009–2010	Hospitals in 11 public sectors, >85 private sectors

Source: Toth,R., Mora-Montes, H., Gabaldon, T.; ***Candida parapsilosis*: from Genes to the Bedside**; Clin.Micro. Rev., 27 February 2019

Candida parapsilosis

High

Clinical Laboratory Diagnostics

Candida parapsilosis

Microscopic

- 10% KOH wet mount and calcofluor stains for observation of fungal **pseudohyphae** under a microscope

Culture

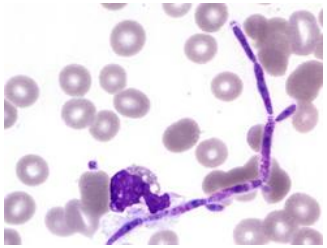
- **SDA** medium to observe white, creamy, shiny, and smooth or wrinkled colonies, with tiny blastospores on a mycelial stalk
- **CMA** = best for pseudohyphae
- **PDA** = white, creamy, shiny, and smooth or wrinkled colonies with larger blastospores

Biochemical characterization

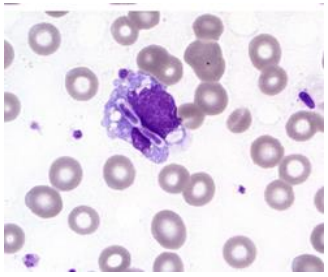
- For detection of urease production and secretion of hydrolyzing enzymes such as phospholipases.

Molecular Assays

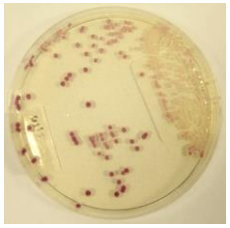
- **PCR** for identification and detection of the fungal genome
- Genomic sequencing to distinguish between various candidal groups from samples.



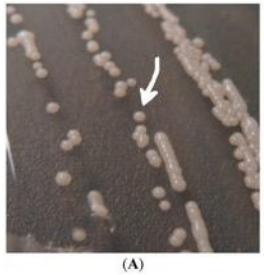
Peripheral blood film (May–Grünwald–Giemsa stain, ×100 objective) = extracellular branched pseudohyphe



Can. paropsilosis = phagocytized

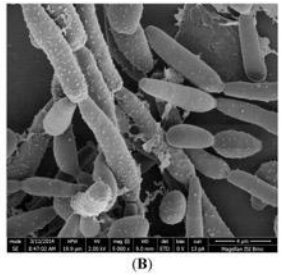


Candida parapsilosis on CHROMAgar™



(A)

SDA, 48 hr. = 2 mm; colonies



(B)

SEM 48 hr. (glass substrate) chemical fixation and freeze-drying (ACE600 Leica microsystems)

Source: Mokobi,F.; ***Candida parapsilosis*- An Overview**; Microbe Notes, 5/6/22

Source: Fenomanana,J, harzallah,I., Lohmann,C., et.al.; **Intracellular yeasts in a peripheral blood film leads to a diagnosis of *Candida parapsilosis* fungaemia**; Br.J.Hem.; 02 August 2020

Pichia kudriavzevii

Medium

Anamorph

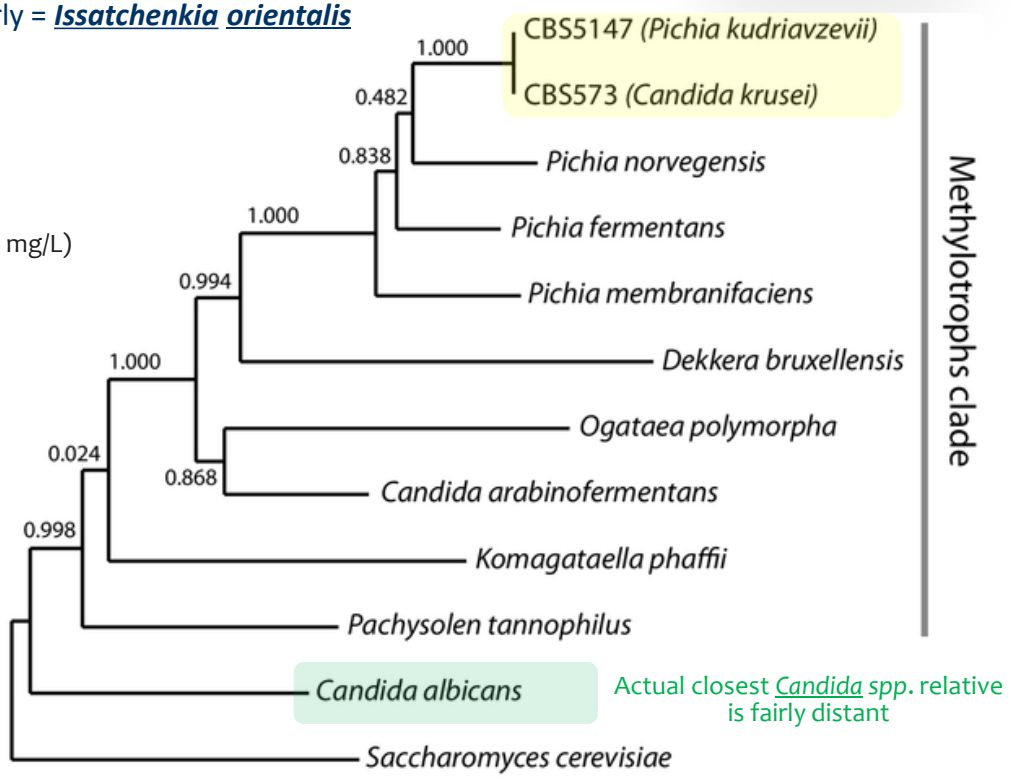
Candida krusei

Pichia kudriavzevii = 99.6% identical DNA

Telomorph

Formerly = ***Issatchenkia orientalis***

- = ~ 2% of yeast infections by *Candida* species in humans
- CVS infections with *Can. krusei* = problematic
  - Most isolates = fluconazole-resistant (**MIC** ≥ 8 mg/L)
    - ∴, likely one gene instead of two at the *ABC11*-*ABC1* tandem locus
  - *Pichia kudriavzevii*, *Issatchenkia orientalis* and *Candida glycerinogenes*, this same organism, = industrial-scale production of glycerol, succinate, some fermented foods
- 1980, Kurtzman and colleagues proposed that *Can. krusei* is the anamorph of a species whose teleomorph is *Pichia kudriavzevii*
- USFDA does NOT regard it as a pathogen ?!!
  - Historically found in fermented cassava and cacao in Africa, fermented milk in Tibet and Sudan, and maize beverages in Colombia
  - It is used in starter cultures for sourdough breads, and in starters (daqu) for Chinese vinegar production from wheat
  - It also has potential as a **probiotic**



Actual closest *Candida spp.* relative is fairly distant

Source: Douglass AP, Offei B, Braun-Galleani S, Coughlan AY, Martos AAR, et al. (2018) **Population genomics shows no distinction between pathogenic Candida krusei and environmental Pichia kudriavzevii: One species, four names.** PLOS Pathogens 14(7): e1007138. https://doi.org/10.1371/journal.ppat.1007138

PLOS

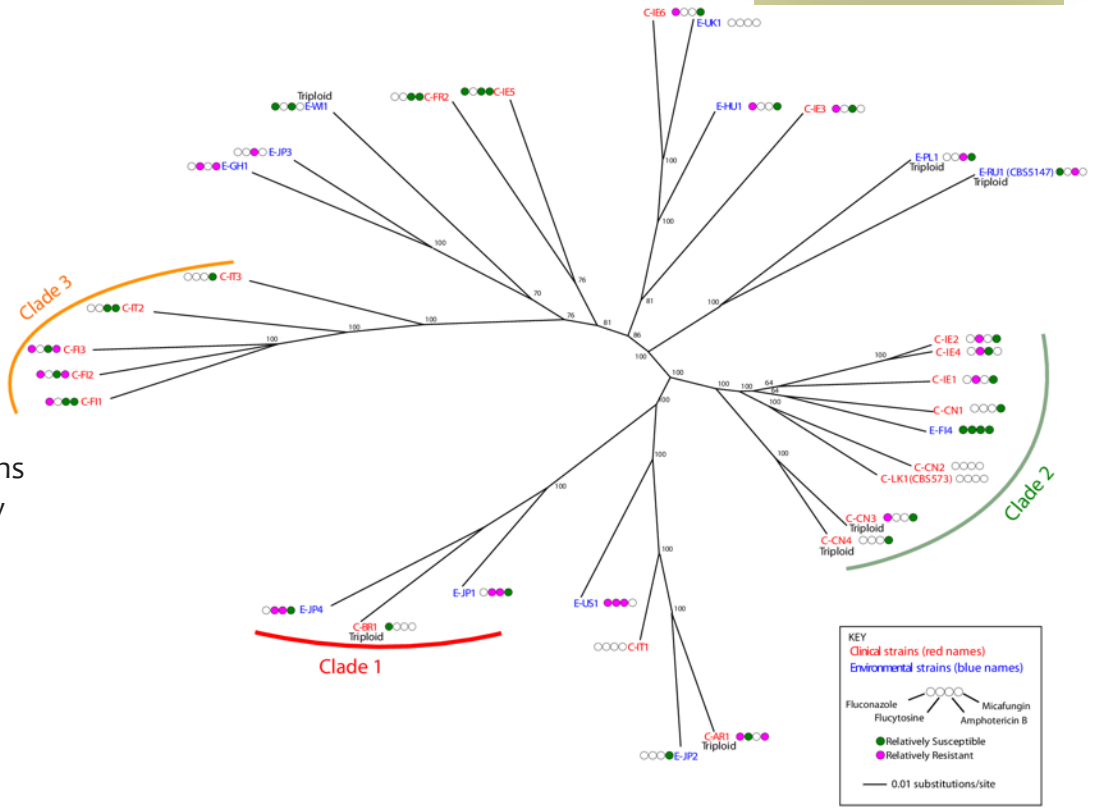
Medium

Pichia kudriavzevii

Candida krusei

Pichia kudriavzevii

- Clinical, environmental strains are NOT segregated into different Clades
  - ∴ mostly acquired by humans from the environment
- Flucytosine = RS and RR strains
- Amphotericin B = RS and RR strains
- Micafungin = More likely to be RS strains
  - MIC ≤ 1 mg/L
- One of more than a dozen NAC reported as a cause candidemia and other invasive infections
- Rapid, reliable identification to species is now needed more than ever for clinicians to make treatment choices



Source: Douglass AP, Offei B, Braun-Galleani S, Coughlan AY, Martos AAR, et al. (2018) **Population genomics shows no distinction between pathogenic *Candida krusei* and environmental *Pichia kudriavzevii*: One species, four names.** PLOS Pathogens 14(7): e1007138. https://doi.org/10.1371/journal.ppat.1007138

Pichia kudriavzevii

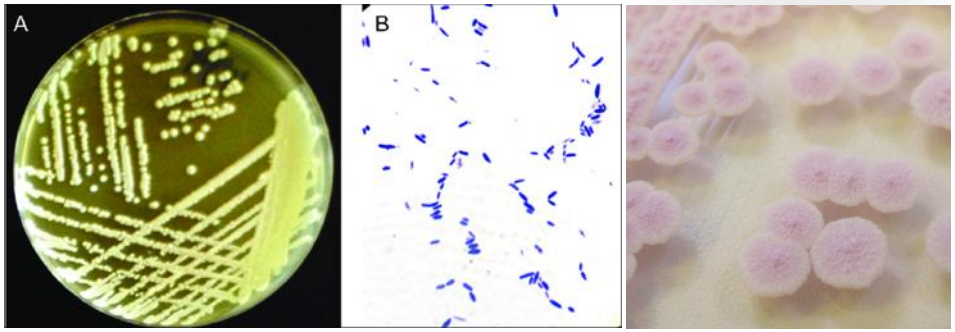
Medium

Clinical Laboratory Diagnostics

Candida krusei

Pichia kudriavzevii

- Most traditional clinical mycology identification techniques are not very helpful per se;
  - Germ Tube -
  - Carbohydrate Assimilation
    - Glucose +
    - Almost all others -
  - Carbohydrate Fermentation
    - Glucose +
    - Almost all others -
  - Chlamydospore Formation -
  - Urea -
- MALDI-TOF MS
- Bichro-latex albicans (Fumouze Diagnostics)
- Krusei color test (Fumouze Diagnostics)
- CHROMagar Candida = rough col. with Pink center, White boarder



(A) SDA @ 5 da., 30°C = showing yeast-like colonies, smooth, glabrous in texture and creamy yellow

CHROmagar

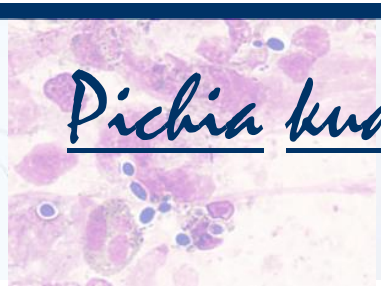
(B) Mx. of the yeast showing gram positive mixtures of elongated cylindrical and spheroidal shaped blastoconidia + Pseudohyphae, + multilateral budding lined up in a **crossed matchsticks** arrangement

- **Mx**, may be confused with ***Magnusiomyces capitatus*** (formerly *Blastoschizomyces capitatus*)
- **Biochemically** need to be carefully distinguished from ***Can. inconspicua*** and ***Can. norvegensis***

Source: Bader,O., Weig,M., Taverne-Gahadwal, L., et.al.; ***Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry;*** Clinical Microbiology and Infection; 17(9),September 2011, Pages 1359-1365

Pichia kudriavzevii

Medium



Clinical Laboratory Diagnostics

Species	**Colony characteristics** on CMA	Morphologic features on CTA	Identification with CMA plus CTA	Identification by **API 20C AUX** or **API 32C** system
Can. albicans	Apple green colonies; consistent distinctive appearance	Chlamydospores present, although >2 days required for some strains; abundant pseudohyphae, some true hyphae, clusters of blastospores along pseudohyphae; distinctive appearance	Accurate identification with both	Identified one strain as *Can. parapsilosis*
Nak.. glabrata	Large pale pink to purple glossy colonies	No pseudohyphae or hyphae; yeast cells are ~2.5 × 4 µm; consistent distinctive appearance	Accurate identification, morphology essential	Identified all strains as *Can. glabrata*
Can. tropicalis	Dull blue, sometimes pink, colonies; all developed purple halo of pigment that diffused into surrounding agar; distinctive appearance	Abundant pseudohyphae often radiating with clusters of blastoconidia at the center; variable appearance	Accurate identification with CMA	Identified all strains as *Can. tropicalis*
Pic. kundriavzevii	**Large, flat, spreading, pale pink colonies** with matt surfaces; **distinctive** appearance	Extensive branched pseudomycelium with chains of elongate cells giving tree-like appearance; clusters and chains of blastospores along pseudohyphae; consistent distinctive appearance (Crossed Matchsticks)	Accurate identification with both	Strains identified as *Can. krusei* with **50%** confidence
Can. parapsilosis	Off-white to pale pink colonies; variable appearance	Branched chains of elongated cells with clusters of blastospores along them; occasional giant cells; variable appearance	Not always able to identify	Identified all strains as *Can. parapsilosis*
Pic. guilliermondii	Small pink to purple colonies; variable appearance	Pseudohyphae with clusters of blastospores; variable appearance	Not always able to identify	API 32C identified strains with **53.7%** confidence
Can. dubliniensis	Dark green colonies	Abundant chlamydospores present; abundant pseudohyphae, some true hyphae, clusters of blastospores along pseudohyphae	Identification with both for the strain examined	Not in API database; identified as *Candida sake* with low confidence

Source: Koehler,A.P., Chu, K.C., houang, E.T.S., et. Al.;m ; ***Simple, Reliable, and Cost-Effective Yeast Identification Scheme for the Clinical Laboratory***, J. Clin. Microb., Feb., 1999,m Vol. 37, No. 2

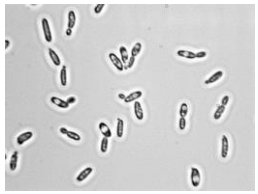
Pichia kudriavzevii

Medium

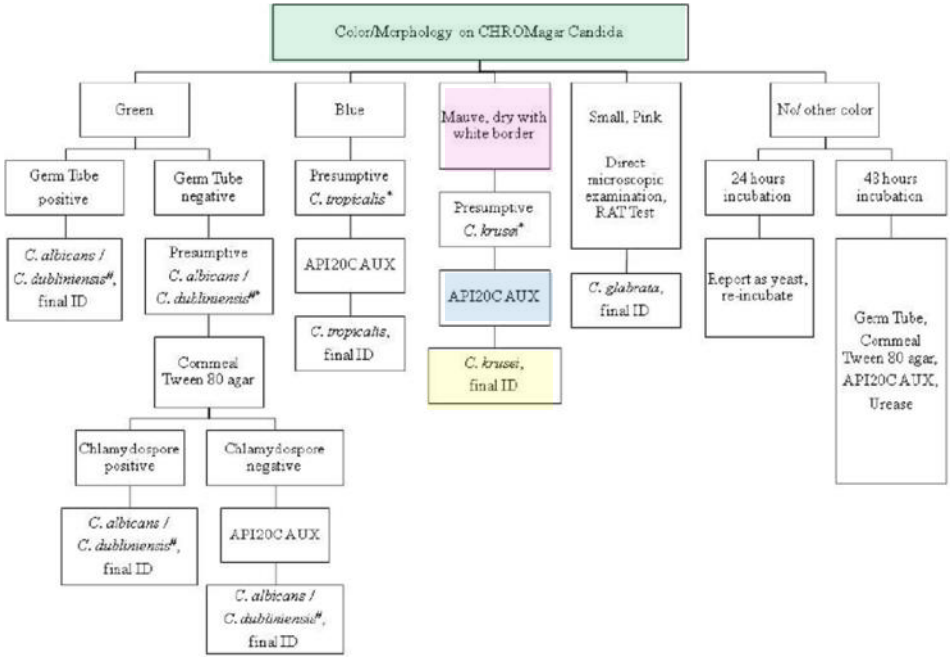
Clinical Laboratory Diagnostics

Candida krusei

Pichia kudriavzevii



CHROmagar



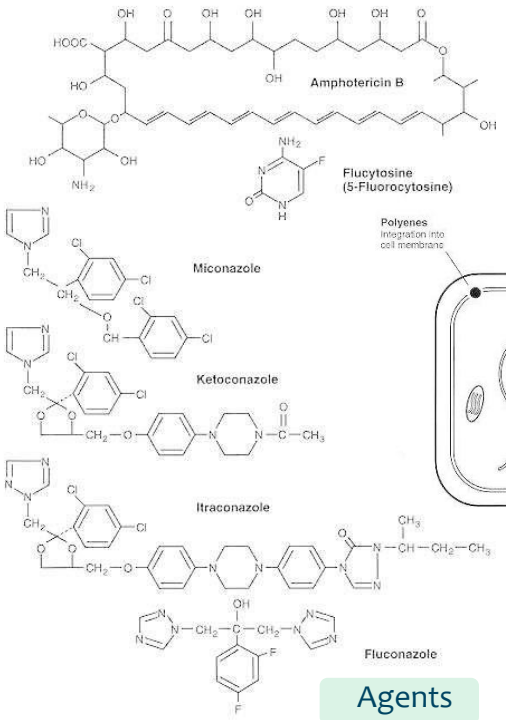
Source: Adam,H.J., Richardson,S.E., Roscoe,M., et.al.; *An Implementation Strategy for the Use of Chromogenic Media in the Rapid, Presumptive Identification of Candida Species*, The Open Mycology Journal, 2010/01/01

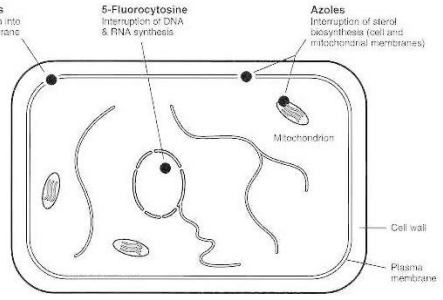
CMM Current Medical Mycology

Antifungals

- A new class of antifungals reaches the market only every 20 years or so:
- The polyene class, including amphotericin B, in the 1950s;
- The azoles in the 1980s;
- The echinocandin drugs, the newest remedy, beginning in 2001
- (There is also terbinafine, used mostly for external infections, and flucytosine, used mostly in combination with other drugs.)
- Olorofim (UK) est. 2023

- Some common antifungals & MOAs;;





- The **best counter** to the ravages of fungi is not treatment but **prevention**: = Vaccines, but we do not have any !!!
- The reason that rates of Valley Fever are not worse than they are, when 10 percent of the U.S. population lives in the endemic area, is that infection confers lifelong immunity
- Since the 1940s researchers have been trying. A prototype that used a killed version of the form *Coccidioides* takes inside the body—fungal spheres packed with spores—worked brilliantly in mice. But it failed dismally in humans in a clinical trial in the 1980s

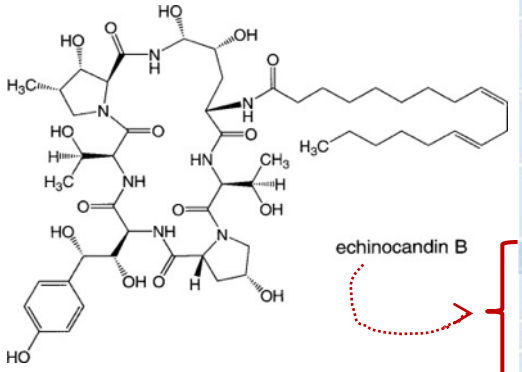
  Dog approval, simple, quick,

  Humans = 5-7 years, $150 Million

  2018, the CDC identified cases of Valley fever in 14 states outside the endemic zone. Most were in wintertime inhabitants of the Southwest who were diagnosed after they went back home.
- By one estimate, a vaccine could save potentially **$1.5 billion** in health-care costs every year

Source: ***WHO fungal priority pathogens list to guide research, development and public health action***. Geneva: World Health Organization; 2022. License: CC BY-NC-SA 3.0 IGO

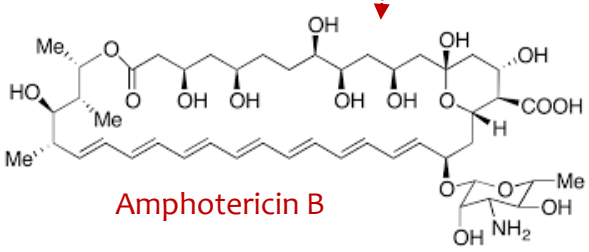
- **Leading Groups** of Antifungal Agents
  - Allylamines
  - Antimetabolites
  - Imidazoles
  - Triazoles
  - Echinocandins



Antifungal Agents	Route	Mechanism of Action	Comments
Allylamines			
Naftifine	Topical	Inhibition of squalene epoxidase	Terbinafine has very broad-spectrum activity and acts synergistically with other antifungals
Terbinafine	Oral, topical		
Antimetabolite			
Flucytosine	Oral	Inhibition of DNA and RNA synthesis	Used in combination with amphotericin B and fluconazole; toxicity and secondary resistance are problems
Imidazoles			
Ketoconazole, bifonazole, clotrimazole, econazole, miconazole, oxiconazole, sulconazole, terconazole, tioconazole	Oral, topical	Inhibits lanosterol 14-α-demethylase cytochrome P450–dependent enzymes	Ketoconazole has modest broad-spectrum activity and toxicity problems
Triazoles			
Fluconazole	Oral, IV	Same as imidazoles but more specific binding to target	Limited spectrum (yeasts); good central nervous system penetration; good in vivo activity; primary and secondary resistance seen with *C. krusei* and *C. glabrata*, respectively
Itraconazole	Oral	Same as imidazoles but more specific binding to target enzyme	Broad-spectrum activity; erratic absorption; toxicity and drug interactions are problems
Voriconazole	Oral, IV	Same as imidazoles but more specific binding to target enzyme	Broad spectrum including yeasts and molds; active vs. *C. krusei*; many drug interactions
Posaconazole	Oral	Same as imidazoles but more specific binding to enzyme	Broad spectrum including activity vs. Zygomycetes
Ravuconazole	Oral, IV	Same as imidazoles but more specific binding to target enzyme	Investigational; broad spectrum including yeasts and molds
Isavuconazole	Oral, IV	Same as imidazoles but more specific binding to target enzyme	Investigational, broad spectrum including activity vs. Zygomycetes
Echinocandins			
Caspofungin	IV	Inhibition of fungal cell wall glucan synthesis	Fungicidal activity against *Candida*
Micafungin	IV		
Anidulafungin	IV		

Source: **Antibiotics in Laboratory Medicine, 6 Ed.; Chapter 6. Antifungal Drugs: Mechanisms of Action, Drug Resistance, Susceptibility Testing, and Assays of Activity in Biologic Fluids by** George R. Thompson III and Thomas F. Patterson

- **Leading Groups** of Antifungal Agents
  - Polyenes
  - Chitin Synthesis Inhibitors
  - others



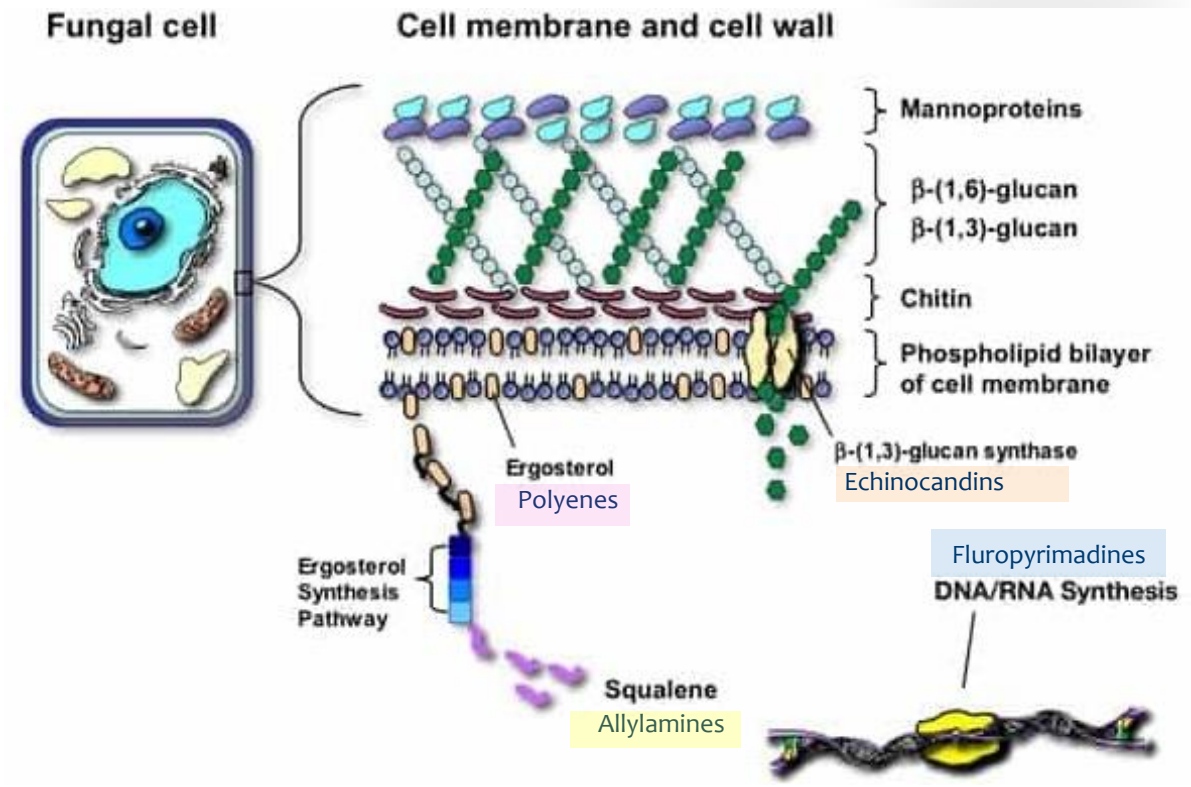
Amphotericin B

Antifungal Agents	Route	Mechanism of Action	Comments
Polyenes			
Amphotericin B	IV, topical	Binds to ergosterol, causing direct oxidative membrane damage	Established agent; broad spectrum; toxic
Lipid formulations (amphotericin B lipid complex or colloidal dispersion, liposomal amphotericin B)	IV	Same as amphotericin B	Broad spectrum; less toxic, expensive
Nystatin	Oral suspension, topical	Same as amphotericin B	Liposomal formulation (IV) under investigation
Natamycin	Topical		Typically used as adjunctive therapy for fungal keratitis
Chitin synthesis inhibitor			
Nikkomycin Z	IV	Inhibition of fungal cell wall chitin synthesis	Investigational agent; possibly useful in combination with other antifungals
Other			
Amorolfine	Topical	Miscellaneous, varied	
Butenafine HCl	Topical		
Ciclopirox olamine	Topical		
Griseofulvin	Oral		
Haloprogin	Topical		
Tolnaftate	Topical		
Undecylenate	Topical		

Source: **Antibiotics in Laboratory Medicine, 6 Ed.; Chapter 6. Antifungal Drugs: Mechanisms of Action, Drug Resistance, Susceptibility Testing, and Assays of Activity in Biologic Fluids by** George R. Thompson III and Thomas F. Patterson

Antifungals

- **Antifungal Targets** (by Class)



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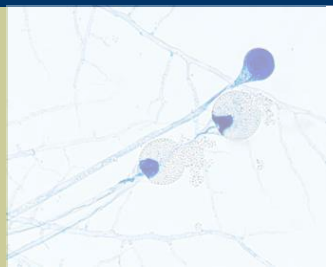

- Representative **Effectiveness**
  - Vs. some common organisms
  - **+** = Antifungal Activity
  - **-** = None likely
- General "effectiveness" suggests that we NEED identification !!!

Organism	AMB	FLU	ITR	POS	VOR	ANI	MFG	CAS	5FC
Aspergillus fumigatus	+	–	+	+	+	+	+	+	–
Aspergillus flavus	+/–	–	+	+	+	+	+	+	–
Aspergillus terreus	–	–	+	+	+	+	+	+	–
Aspergillus niger	+	–	+/–	+	+	+	+	+	–
Aspergillus nidulans	+	–	+/–	+	+	+	+	+	–
Candida albicans	+	+	+	+	+	+	+	+	+
Candida glabrata	+	+/–	+/–	+/–	+/–	+	+	+	+
Candida krusei	+	–	+/–	+	+	+	+	+	+/–
Candida tropicalis	+	+	+	+	+	+	+	+	+
Candida parapsilosis	+	+	+	+	+	+/–	+/–	+/–	+
Candida guillermondii	+	+	+	+	+	–	–	–	+
Candida lusitaniae	–	+	+	+	+	+	+	+	+
Cryptococcus spp	+	+	+	+	+	–	–	–	+
Blastomyces	+	+	+	+	+	+/–	+/–	+/–	–
Histoplasma	+	+/–	+	+	+	+/–	+/–	+/–	–
Coccidioides	+	+	+	+	+	–	–	–	–
Sporothrix	+	–	+	+/–	–	+/–	+/–	+/–	–
Fusarium spp	+/–	–	–	+	+	–	–	–	–
Phaeohyphomycoses[a]	+	–	+	+	+	+	+	+	–
Pichia spp	+	+	+/–	+	+	+	+	+	+
Saccharomyces spp	+	+	+	+	+	+	+	+	+
Scedosporium apiospermum	+/–	–	+/–	+	+	–	–	–	–
Scedosporium prolificans	–	–	–	+/–	+/–	–	–	–	–
Trichosporon spp	+/–	+	+	+	+	–	–	–	+
Mucorales	+/–	–	–	+	–	–	–	–	–

Source: **Antibiotics in Laboratory Medicine, 6 Ed.; Chapter 6. Antifungal Drugs: Mechanisms of Action, Drug Resistance, Susceptibility Testing, and Assays of Activity in Biologic Fluids by** George R. Thompson III and Thomas F. Patterson

Common Mechanisms of Resistance

Fungus	Amphotericin B	Flucytosine	Itraconazole/Voriconazole	Fluconazole	Echinocandins
Aspergillus fumigatus			Altered target enzyme, 14-α-demethylase Decreased azole accumulation (TR/L98H) promotor mutation, HapE transcription factor mutations		
Candida albicans	Decrease in ergosterol Replacement of polyene-binding sterols Masking of ergosterol	Loss of permease activity Loss of cytosine deaminase activity Loss of uracil phosphoribosyl-transferase activity		Overexpression or mutation of 14-α-demethylase Overexpression of efflux pumps, *CDR* and *MDR* genes	Mutation in *FKS1/2* genes
Candida glabrata	Alteration or decrease in ergosterol content	Loss of permease activity		Overexpression of efflux pumps (*CgCDR* genes)	Mutation in *FKS1/2* genes
Candida krusei	Alteration or decrease in ergosterol content			Active efflux Reduced affinity for target enzyme, 14-α-demethylase	Mutation in *FKS1/2* genes
Candida lusitaniae	Alteration or decrease in ergosterol content Production of modified sterols				
Cryptococcus neoformans	Defects in sterol synthesis Decreased ergosterol Production of modified sterols			Alterations in target enzyme Overexpression of MDR efflux pump	

Source: **Antibiotics in Laboratory Medicine, 6 Ed.; Chapter 6. Antifungal Drugs: Mechanisms of Action, Drug Resistance, Susceptibility Testing, and Assays of Activity in Biologic Fluids by** George R. Thompson III and Thomas F. Patterson

- **M**echanism **o**f Action

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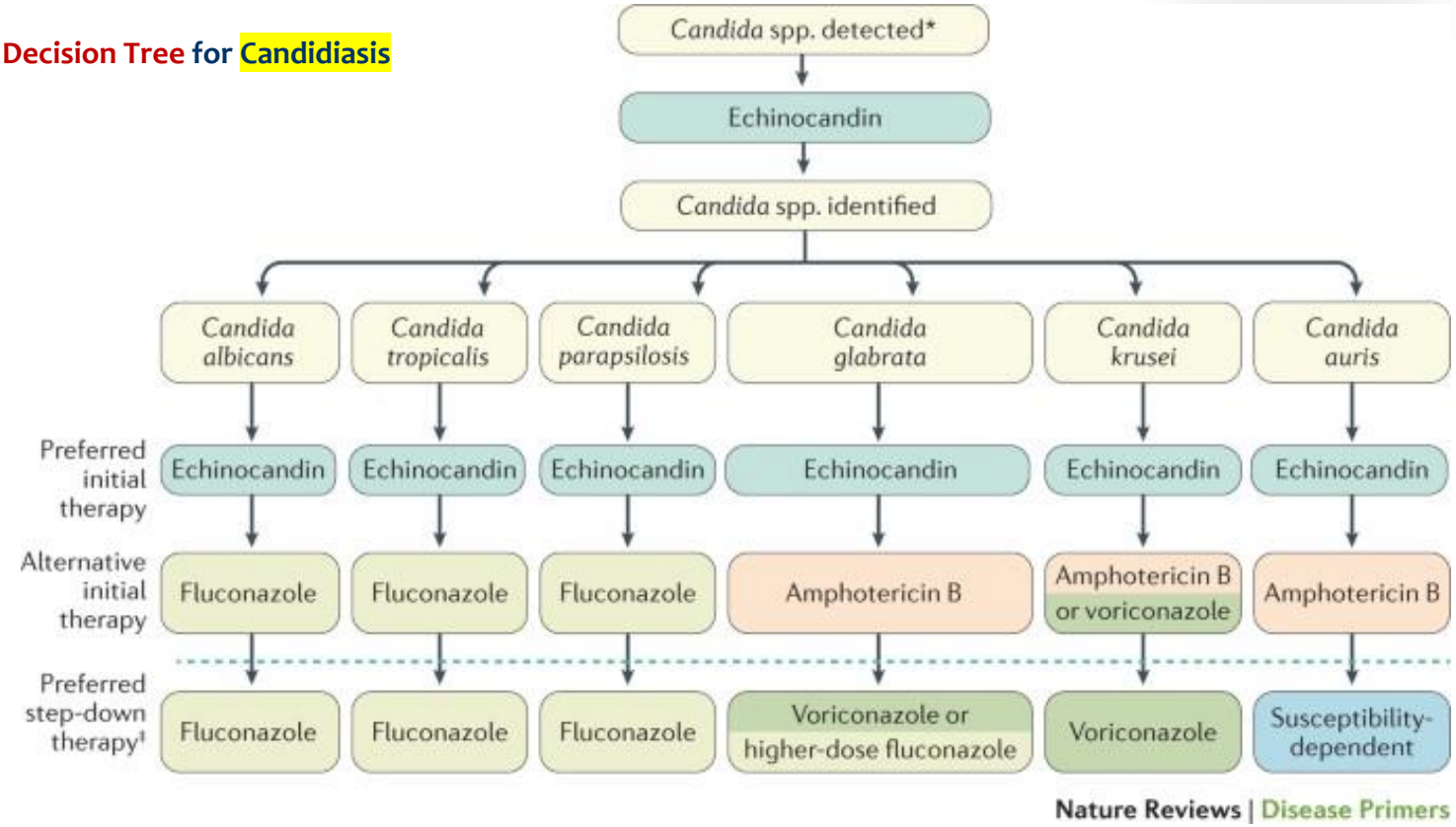
Nystatin	Oral suspension, topical	Same as amphotericin B	Liposomal formulation (IV) under investigation
Natamycin	Topical		Typically used as adjunctive therapy for fungal keratitis
Chitin synthesis inhibitor			
Nikkomycin Z	IV	Inhibition of fungal cell wall chitin synthesis	Investigational agent; possibly useful in combination with other antifungals
Other			
Amorolfine	Topical	Miscellaneous, varied	
Butenafine HCl	Topical		
Ciclopirox olamine	Topical		
Griseofulvin	Oral		
Haloprogin	Topical		
Tolnaftate	Topical		
Undecylenate	Topical		

Source: **Antibiotics in Laboratory Medicine, 6 Ed.; Chapter 6. Antifungal Drugs: Mechanisms of Action, Drug Resistance, Susceptibility Testing, and Assays of Activity in Biologic Fluids by** George R. Thompson III and Thomas F. Patterson

Antifungals

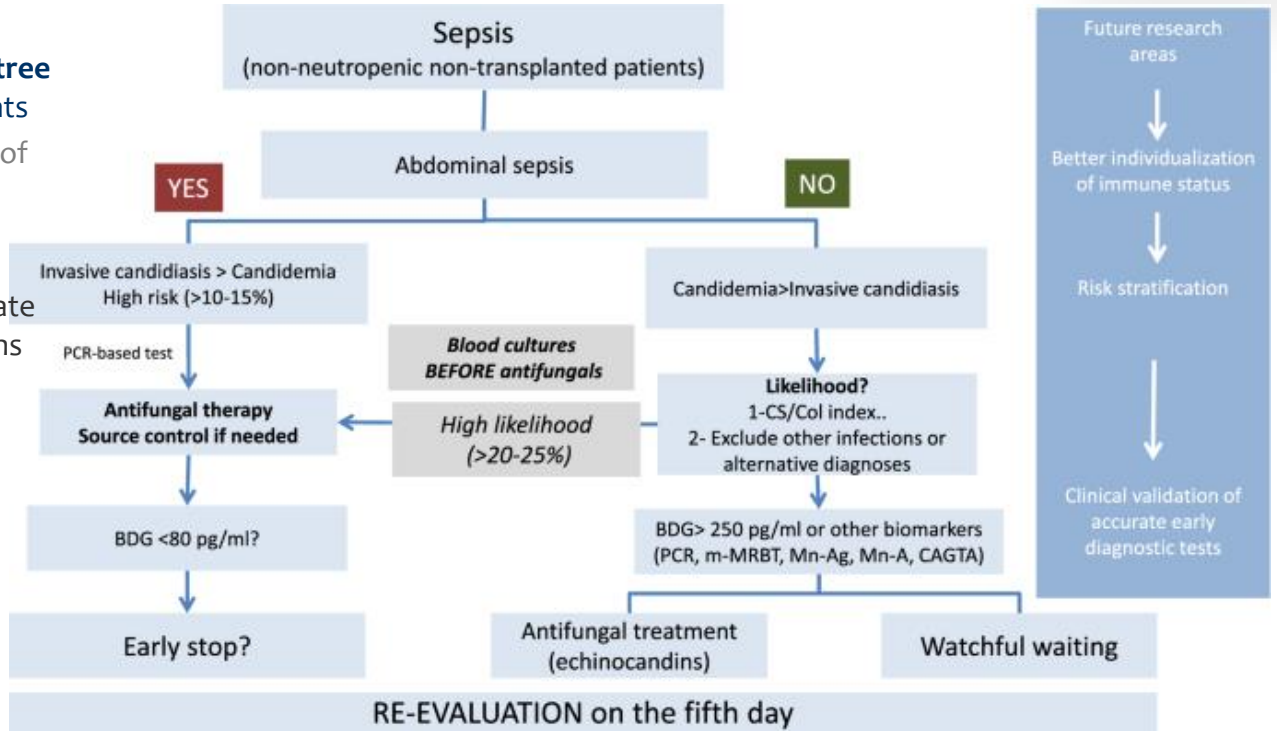
- **Treatment Decision Tree for Candidiasis**



Source: Pappas,P.G., Lionakis,M.S., Arendrup, M.C., et.al.; ***Invasive Candidiasis***; Nat. Rev.; 18026, 11 May 2018

Antifungals

- Treatment **decision tree** for critically ill patients
- Via GRADE (Grading of Recommendations Assessment, Development, and Evaluation) to evaluate the recommendations and assign levels of evidence
- > 80% agreement = consensus



Source: Martin-Loeches, I, Antonelli, M., Cuenca-Estrella, M.,et.al.; ***ESICM/ESCMID task force on practical management of invasive candidiasis in critically ill patients***; *Intensive Care Medicine* **volume 45**, pages 789–805 (2019)

Suggested Drugs for **Treatment of Invasive Candidiasis**
Amphotericin B (AmB)
Lipid formulation (LF)
CNS

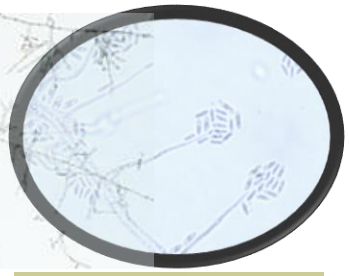
Candidemia Characteristic	Treatments		
	Primary	**Alternative**	**New Drugs**
Non-Neutropenic patients	Caspofungin Anidulafungin Micafungin	LF AmB Fluconazole * Isavuconazole Voriconazole	Ibrexafungerp
Neutropenic Patients	Caspofungin Anidulafungin Micafungin	AmB Liposomal Fluconazole * Isavuconazole Voriconazole	Rezafungin Osteaconazole
Ocular Compromise +	Fluconazole Voriconazole	AmB Liposomal	Fosmanogepix
CNS Compromise +	AmB Liposomal	Fluconazole	

* Use in stable patients without prior use of azoles; + 6 weeks of treatment

antibiotics

Source: Riera,F.O., Caeiro,J.P., Angiolini, S.C., et.al.; **Invasive Candidiasis: Update and Current Challenges in the Management of This Mycosis in South America;** *Antibiotics* **2022**, *11*(7), 877, **30 June 2022**

WHO Guidelines for Next Steps; Clinical Laboratories are Critical



- **Build mycology diagnostic capacity** to manage fungal infections and to perform **surveillance**, starting at reference microbiology laboratories for **identification and susceptibility testing** of fungi.
- **Integrate fungal diagnostics** that are included on WHO's model list of essential diagnostics into **routine care** or **specialized laboratories** based on local epidemiology, contexts, capacity and needs. Prioritize diagnostic services to serve **populations at greatest risk** of fungal diseases (e.g., cancer, HIV/AIDS, post-TB, COPD, asthma).
- Build capacity in antifungal stewardship to **limit the inappropriate use of antifungals as well as antibiotics**. Develop **standard operating procedures** and algorithms for laboratories to **optimize the diagnosis** of fungal infections, including for pathogens with outbreak potential; build capacity for outbreak detection, reporting and response.
- Encourage the development of **networks at the national and international level** and participate in collaborative global and regional surveillance initiatives (e.g., WHO GLASS-AMR, GLASS-FUNGI, GLASS-EAR, and other regional platforms such as ReLAVRA and EARS-Net).
- Utilize epidemiological laboratory and clinical **surveillance data** along with other health care data to **quantify** the burden of IFD and **antifungal resistance** to inform public health interventions, and guide IPC measures.
- Follow a stepwise approach in implementing the FPPL beginning with top priority pathogens, starting with **data and evidence generation**, and **tailoring FPPL to regional, national, and local contexts and needs**.

19 → 4 Organisms | 7 Organisms | 8 Organisms

Critical group	High group	Medium group
Cryptococcus neoformans	*Nakaseomyces glabrata (Candida glabrata)*	*Scedosporium* spp.
Candida auris	*Histoplasma* spp.	*Lomentospora prolificans*
Aspergillus fumigatus	Eumycetoma causative agents	*Coccidioides* spp.
Candida albicans	Mucorales	*Pichia kudriavzevevii (Candida krusei)*
	Fusarium spp.	*Cryptococcus gattii*
	Candida tropicalis	*Talaromyces marneffei*
	Candida parapsilosis	*Pneumocystis jirovecii*
		Paracoccidioides spp.

Emerged as **MORE** deadly during COVID-19

Source: ***WHO fungal priority pathogens list to guide research, development and public health action***. Geneva: World Health Organization; 2022. License: CC BY-NC-SA 3.0 IGO

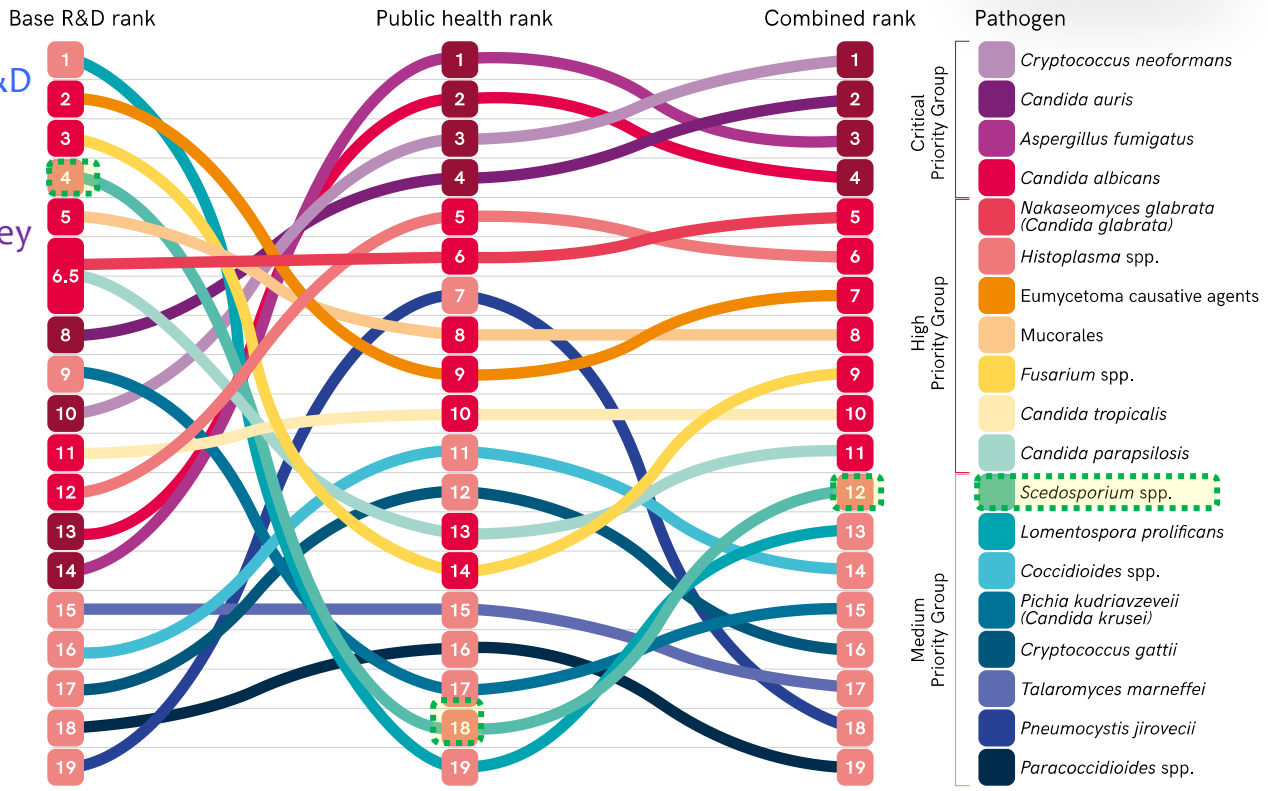
WHO Guidelines for Next Steps; Clinical Laboratories are Critical

- **Intervention**
  - Countries are encouraged to **improve their mycology diagnostic capacity** to manage fungal infections
  - Countries are encouraged to to **optimize** and standardize the use of **current diagnostic modalities**
  - Public health interventions are needed to **highlight the importance of fungal infections**, including through **incorporating fungal diseases and priority pathogens in medical** (clinical) **and public health training programs and curricula at all levels of training**
- **Surveillance**
  - Countries are encouraged to **perform surveillance**
- **Innovation**
  - **More investments are needed in** basic mycology research, R&D of antifungal drugs and **diagnostics**

Source: *WHO fungal priority pathogens list to guide research, development and public health action*. Geneva: World Health Organization; 2022. License: CC BY-NC-SA 3.0 IGO

WHO Fungal Priority Pathogens

- Base R&D Rank
  - From DCE Survey on R&D Priorities
- Pathogen Ranking
  - From BWS Scaling Survey
- Overall Combined Ranking



Mycology of the Near Future

- Improved standards of care depend on the development of new laboratory diagnostic and imaging procedures
  - Immunochromatography technologies have led to the development of lateral flow devices for the diagnosis of cryptococcal meningitis and invasive aspergillosis (IA)
  - Similar devices are being developed for the detection of histoplasmosis that meet the requirements for speed (~15 min assay time)
  - The evolution of molecular tools for the detection of fungal pathogens has been slow but the introduction of new nucleic acid amplification techniques appears to be helpful
    - T2Candida
    - MRI with T2-weighted turbo-spin-echo sequences exhibits sensitivity and specificity approaching that of CT for the diagnosis of invasive pulmonary aspergillosis
  - An Aspergillus proximity ligation assay has been developed for a rapid near-patient bedside diagnosis of IA
  - CT remains the cornerstone for radiological diagnosis of invasive pulmonary fungal infections. MRI of the lungs may be performed to avoid radiation exposure

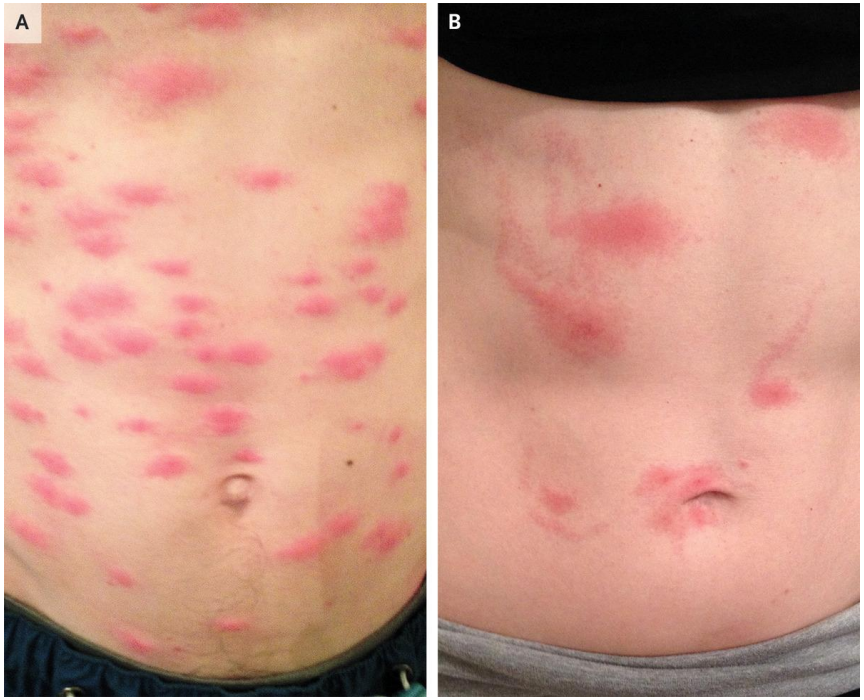
Source: Sanguinetti,M., Posteraro,B., Beigelman-Aubry,C., et.al.; **Diagnosis and treatment of invasive fungal infections: looking ahead**
J Antimicrob Chemother. 2019 Mar 1;74(Suppl 2):ii27-ii37

Quiz

- A 30-year-old man and a 30-year-old woman presented to the infectious disease clinic with a 3-day history of itchy skin lesions
  - No history of outdoor activities
  - Man had pruritic papules across his trunk and abdomen
  - On the woman's abdomen, there were several erythematous macules, some of which had central dots and serpiginous tracts emanating from them that gave the appearance of a comet
- Owing to concern about a household infestation, the couple's furniture was examined

  - *Anobium punctatum*, (wood-boring furniture beetle)
  - *Parasitized by Pyemotes ventricosus*, (European straw itch **mite**)

  - Treatment with topical glucocorticoids and antihistamines
    - Resolution in 8 days



Source: Berenger,J.M., Parola, P; Aix-Marseille University, Marseille, France, New England J. Med., 6/24/23

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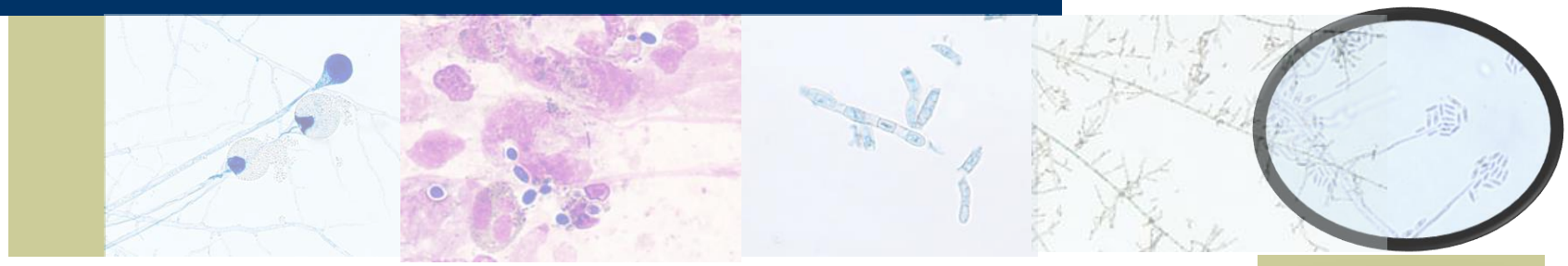

Questions