"Clinical Impact of Antifungal Resistance among *Candida* and *Aspergillus* species"

Jose A. Vazquez, M.D., FACP, FIDSA
Professor of Medicine
Chief, Division of Infectious Diseases
Chair, Antimicrobial Stewardship Program
Augusta University/Medical College of Georgia
Augusta, GA

Conflict of Interest

Consultant: Cidara, F2G, Scynexis, Pfizer

Research Support: Next Science

"All experts agree that by the year 2000, viral and bacterial disease will have been eradicated."

Time 2/66

Objectives

A. Describe the various types of resistance mechanisms in antifungals
B. Describe the different types of fungal pathogens and their resistance
C. Define antifungal resistance and breakpoints
D. Describe how to manage MDR fungal pathogens

“Fun Fungal Facts”

- Ancient – existed 300 million years ago
- Over 2 million species and 100,000 varieties
- Free living: not dependent on humans or animals
- Most have limited pathogenicity
- Positive attributes – edible fungi, yeasts, alcoholic beverages, penicillin

Pop Quiz - 1

- There are over 150 *Candida* spp., the following are in the top 5 of Candida species isolated:
  a) *Candida albicans*
  b) *C. glabrata*
  c) *C. krusei*
  d) *C. dubliniensis*
  e) *All of the above*
  f) A+B+C

Pop Quiz - 1

- There are over 150 *Candida* spp., the following are in the top 5 of species isolated:
  a) *Candida albicans (most common)*
  b) *C. glabrata – 2nd*
  c) *C. krusei - 5th*
  d) *C. dubliniensis (OPC in HIV) > 90%*
  e) *All of the above*
  f) A+B+C

What has changed over the last 5 years?

1. Increase incidence of invasive fungal infections
2. Continued drift to non *albicans Candida* spp.
3. Shifting azole & candin resistance (*C. glabrata, C. parapsilosis*)
4. Increased antifungal resistant fungi (C. auris & C. parapsilosis)
5. Increased use of echinocandins (IV)
    a. Echinocandin resistance (Rapid S to R)
6. Increased "De-escalation policies"

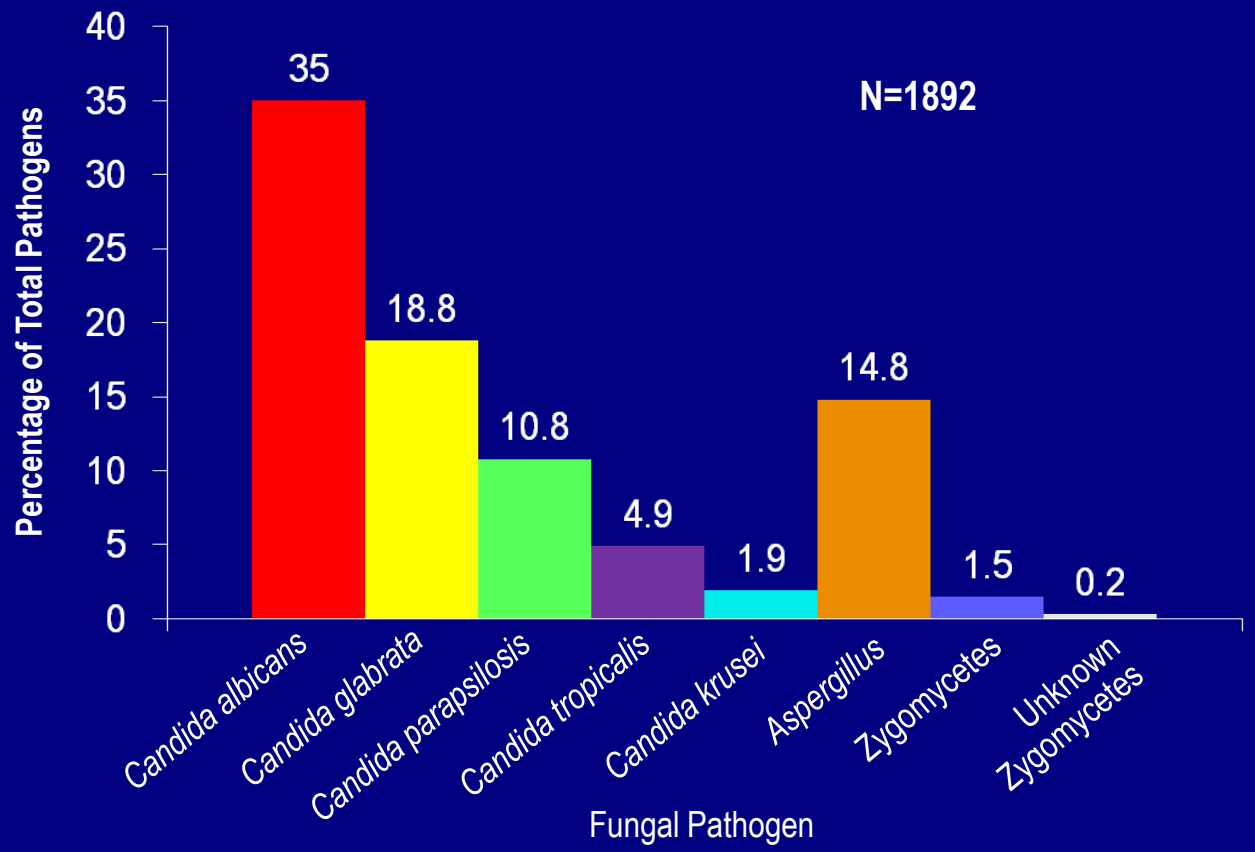
Fungal Burden -2019

Disease	Hospitalizations[a]	Outpatient Visits[a]	Average Workdays Lost per Hospitalization[b]	Proportion of Nonfatal Hospitalizations Among Working-Age Patients[c]	Deaths[d]
Aspergillosis	14 820	[e]	20.3	52%	723
Blastomycosis	950	[e]	13.2	68%	60
Candida infection					
Invasive candidiasis	12 770	[e]	28.4	50%	655
Noninvasive candidiasis	13 990	3 639 037	5.9	42%	537
Coccidioidomycosis	6670	[e]	13.4	67%	192
Cryptococcosis	4755	[e]	19.7	70%	334
Dermatophytosis	690	4 981 444	3.7	65%	36
Histoplasmosis	4630	79 993	13.4	61%	133
Pneumocystis pneumonia	10 590	[e]	16.6	78%	436
Mucormycosis	1140	[e]	25.6	61%	134
Other and unspecified mycoses	7355	222 523	22.1	57%	1645

Economic Burden: ~ $11.5 -50 billion

Benedict K, et al. OFID March 2022 doi.org/10.1093/ofid/ofac097

Epidemiology of Invasive Fungal Infections: PATH Alliance Registry



Horn DL et al. *Diagn Microbiol Infect Dis*. 20017;59:407-414.

Resistance

In vitro resistance vs clinical resistance ??

Case 1

55 year old male attorney underwent Colectomy for Dukes A colon CA

Day 2 post op:	Antibiotics started for suspected post-op wound infection
Day 6:	New fever B.C. drawn
Day 8:	B.C. (+) for *C. glabrata* in Bactec bottles 1/4
	Central venous catheter removed
	Afebrile after 24 hours
	Repeat B.C. (-)
Day 10:	Pt. d/c home on Fluconazole 400mg daily

Patient (attorney) returns one month later with complaining of decrease visual acuity in left eye.

Could there be a relationship between his eye symptoms and his prior hospitalization?

1. Yes, absolutely
2. Possibly related
3. No
4. I don’t know.

Patient (attorney) returns one month later with complaining of decrease visual acuity in left eye.

Could there be a relationship between his eye symptoms and his prior hospitalization?

1. Yes, absolutely
    1. * *C. glabrata* has high levels of intrinsic resistance to fluconazole, thus echinocandins are the correct choice until MICs are done.
2. Possibly related
3. No
4. I don’t know.

Candida Infections

Vazquez, J. Lab

Significance of Candidal Infections

- *Candida* species represent the most common cause of systemic fungal infections & a major cause of M/M among compromised host
- Sepsis due to fungi has increased by > 200% in past 20 yrs
- Outcomes attributable to candidemia/candidiasis
  - Hospital cost ~ $ 44 – 320 million/yr
  - Increase LOS up to 34 days
  - 36-63% mortality
- However, the true incidence is still underestimated & is frequently a post-mortem diagnosis

Fridkin S, CID. 2005;41:1455-60. Martin G, NEJM 2003;348:1546-54. Rentz AM, CID 1998; 27:781-8. Wey S. Arch Intern Med 1988;148:2642-5. Fraser j. CID. 1992;15:414-421.

Predominant Pathogens in BSI: SCOPE STUDY

Rank	Pathogen	2000 (%)	2014 (%)	Mortality (%)
1	Coag. neg *Staphylococcus*	31.9	31.3	21
2	*S. aureus*	15.7	20.2	26
3	*Enterococcus*	15.7	9.4	34
4	***Candida* spp.**	**7.6**	**9.0**	**39**
5	*E.coli*	5.7	5.6	22
6	*Klebsiella*	5.4	4.8	28
7	*Pseudomonas*	< 1.0	4.3	39
8	*Enterobacter*	4.5	3.9	27

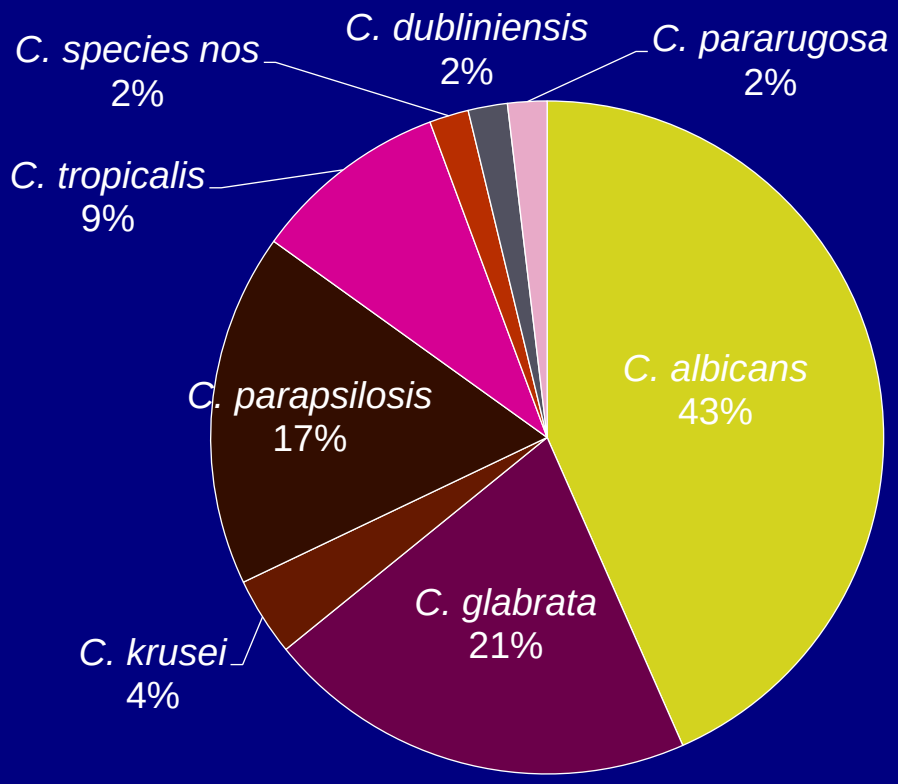
Edmond M et al. CID 1999;29:239 & Wisplinghoff et al. CID 20014;39:309-17.

CANDIDA SPP.
Why Should They Be Identified?

- *C. albicans*
- *C. glabrata* - < susceptible to all antifungals
- *C. parapsilosis* - catheter related
  - (candin resistance ??)
- *C. tropicalis*
- *C. krusei* - “neutropenics”- intrinsic azole resistance, less susceptible - resistant to AmB
- *C. dubliniensis* predominantly in HIV
- *C. guillermondi*
- *C. lusitaniae* } AMB resistance
- *C. auris* *MDR*

> 150 species of *Candida* in nature

Distribution of *Candida* species Causing Candidemia: AUMC, 2020



Candida species: Antifungal Resistance Implications

- *Candida albicans*
  - *Most common spp isolated*
  - *Rare resistance, except in long-term antifungal use in compromised host*

- *Candida glabrata*
  - Increasing rates of resistance to azoles (30-40% in some centers)
  - Echinocandin resistance increasing slowly
    - Up to 36% also resistant to fluconazole/voriconazole

- *Candida parapsilosis*
  - Higher MIC to echinocandins *in vitro*
    - Clinical significance unclear
  - Increase resistance to fluconazole

Pappas et al 2016; Perlin 2015

Species of Candida: Antifungal Resistance Implications

- *C. tropicalis*
  - < 5% resistance to fluconazole
- *C. krusei*
  - Original MDR yeast
  - Intrinsic resistant to fluconazole/voriconazole/? Posaconazole/?isavuconazole
  - Less susceptible to polyenes
- *C. lusitaniae/guillermondii*
  - Intrinsic resistance to polyenes
  - Switching phenotypes

Pappas et al 2016; Perlin 2015

Candida auris Implications

- *Crude mortality rates 30-60%*
- *MDR, including*
  - *95 % R-flz*
  - *6% R-Flz and AmB*
  - *Rare (R-azole, polyene, candin) < 1%*

- *Thus, candins are drug of choice.*
- *Problem is the environmental re-contamination (tables, bedrails, remotes)- occasionally within 4 hrs of cleaning*
- *Personnel: hands*
- *Patients: colonization of nares & hands ~ 72%*

Maphanga TG. Et al., AAC; doi.org/10.1128/AAC.00517-21: Sansom S et al . Abstract 50, SHEA, April 2022.

Review of Common Antifungals

Azoles :

- Fluconazole
- Itraconazole
- Voriconazole
- Posaconazole
- Isavuconazole

- Echinocandins
  - Caspofungin
  - Micafungin
  - Anidulafungin
- Polyenes
  - AmB
  - Nystatin

Fungistatic vs fungicidal (does it make a difference?)

2016 IDSA/MSG Update: Treatment of Candidemia in Nonneutropenic Patients

- An echinocandin is recommended as initial therapy for all Candida spp.
  - Caspofungin, micafungin, or anidulafungin
  - Dosing dependent on which agent used

- Fluconazole, IV or oral, is an **acceptable alternative** as initial therapy in patients who are:
  - Not critically ill
  - Unlikely to have fluconazole-resistant *Candida* sp.
    - Dose at 800-mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily
    - Adjust for renal function

- Test for azole susceptibility for all bloodstream & other clinically relevant Candida isolates

Consider testing for echinocandin susceptibility if:

- h/o prior treatment with an echinocandin
- Infection with *C. glabrata* or *C. parapsilosis*

Pappas PG et al CID Dec 16th 2015

Antifungal Resistance: Testing

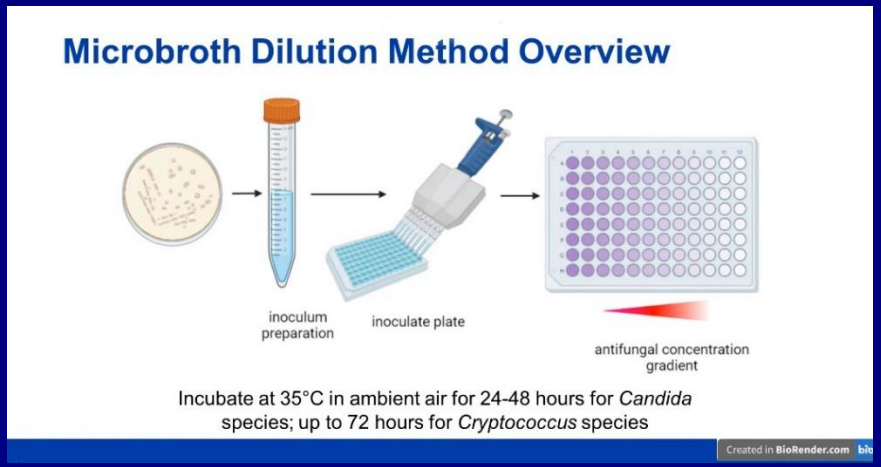
- Reproducible in agar, broth dilution, macro & microdilution
- Performed on Yeast, some moulds

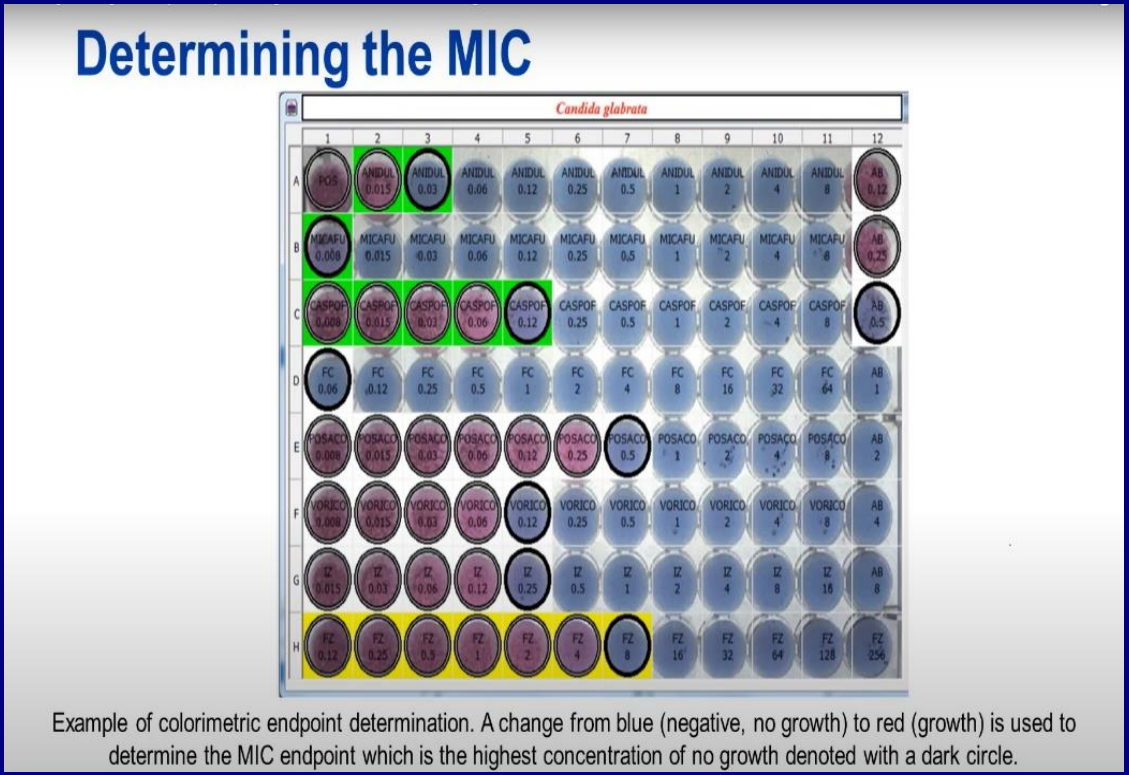
- Susceptibility of *Candida* to the currently available antifungal agents is generally predictable by species
  - Antifungal resistance in *C. albicans* is currently rare
  - *C. glabrata* resistance increasingly common, supporting need for susceptibility testing

- Clinical and Laboratory Standards Institute (CLSI)
  - Published revised standardized testing methods for evaluating antifungal susceptibility in *Candida spp.* & other yeasts using broth dilution in 2012
    - Clinical breakpoints established for most antifungals against the 5 most common Candida species
    - Inter-laboratory variability remain a concern, particularly with caspofungin testing
      - Molecular testing may emerge as important modality for predicting echinocandin resistance

CLSI 2012; Pfaller et al 2011

In Vitro Susceptibility Testing





Sensititre-Plate-Guide-Booklet-EN.pdf (thermofisher.com)

https://clsi.org/standards/products/free-resources/access-our-free-resources/

In vitro Susceptibility

Inoculum Preparation	Broth Type	Use, Methodology and Region	Read Method
0.5 McFarland Standard (E1041) **Sensititre Sterile Water** (T3339)	**Sensititre YeastOne Broth** (Y3462)	**IVD** FDA, CLSI [Worldwide]	**Manual** Sensititre Vizion (V2021) Sensititre Manual Viewbox (V4007)

1. Put 3-5 colonies into H_2O to measure a 0.5 McFarland using the Nephelometer, Mix 20 µl of suspension into Sensititre YeastOne Broth
2. Inoculate plate with 100 µl volume per well of the suspension using the Sensititre AIM or Multi-Channel Pipette
3. Seal Sensititre plate and incubate at 35°C in a non-CO_2 incubator for 24-25 hours
4. Manually read with Sensititre Vizion or Sensititre Manual Viewbox

	1	2	3	4	5	6	7	8	9	10	11	12
A	POS	VOR 0.008	VOR 0.015	VOR 0.03	VOR 0.06	VOR 0.12	VOR 0.25	VOR 0.5	VOR 1	VOR 2	VOR 4	IZ 2
B	FC 0.03	FC 0.06	FC 0.12	FC 0.25	FC 0.5	FC 1	FC 2	FC 4	FC 8	FC 16	IZ 0.03	IZ 1
C	FZ 0.25	FZ 0.5	FZ 1	FZ 2	FZ 4	FZ 8	FZ 16	FZ 32	FZ 64	FZ 32	IZ 0.06	IZ 0.5
D	CAS 0.015	CAS 0.03	CAS 0.06	CAS 0.12	CAS 0.25	CAS 0.5	CAS 1	CAS 2	CAS 4	CAS 8	IZ 0.12	IZ 0.25
E	POS	VOR 0.008	VOR 0.015	VOR 0.03	VOR 0.06	VOR 0.12	VOR 0.25	VOR 0.5	VOR 1	VOR 2	VOR 4	IZ 2
F	FC 0.03	FC 0.06	FC 0.12	FC 0.25	FC 0.5	FC 1	FC 2	FC 4	FC 8	FC 16	IZ 0.03	IZ 1
G	FZ 0.25	FZ 0.5	FZ 1	FZ 2	FZ 4	FZ 8	FZ 16	FZ 32	FZ 64	FC 32	IZ 0.06	IZ 0.5
H	CAS 0.015	CAS 0.03	CAS 0.06	CAS 0.12	CAS 0.25	CAS 0.5	CAS 1	CAS 2	CAS 4	CAS 8	IZ 0.12	IZ 0.25

Antimicrobics

FC	5-Flucytosine
FZ	Fluconazole
CAS	Caspofungin
VOR	Voriconazole
IZ	Itraconazole
POS	Positive control

Sensititre-Plate-Guide-Booklet-EN.pdf (thermofisher.com)

Clinical Breakpoints
What do you do with them ??

Table 1. Clinical Breakpoints for Antifungal Agents Against Common *Candida* Species

		Clinical Breakpoint, μg/mL[a]			
Candida Organism	Antifungal Agent	S	SDD	I	R
C. albicans	Fluconazole	≤2	4		≥8
	Itraconazole	≤0.12	0.25–0.5		≥1
	Voriconazole	≤0.12		0.25–0.5	≥1
	Posaconazole				
	Anidulafungin	≤0.25		0.5	≥1
	Caspofungin	≤0.25		0.5	≥1
	Micafungin	≤0.25		0.5	≥1
C. glabrata	Fluconazole		32		≥64
	Itraconazole				
	Voriconazole				
	Posaconazole				
	Anidulafungin	≤0.12		0.25	≥0.5
	Caspofungin	≤0.12		0.25	≥0.5
	Micafungin	≤0.06		0.12	≥0.25
C. parapsilosis	Fluconazole	≤2	4		≥8
	Itraconazole				
	Voriconazole	≤0.12		0.25–0.5	≥1
	Posaconazole				
	Anidulafungin	≤2		4	≥8
	Caspofungin	≤2		4	≥8
	Micafungin	≤2		4	≥8

Pappas et al. 2016. pg 14

Fungal Factors Affecting Resistance

- Initial MICs of *Candida* spp
- Cell types
  - yeast/hyphal
- Genomic stability
  - Diploid vs haploid
- Size of population
    - population “bottlenecks”
- Protective, inaccessible mucosal environment
  - Biofilm formation (polymicrobial biofilms)

Antifungal Resistance

- Changing epidemiology has important clinical implications
  - Non-*albicans* species more likely to have resistance to fluconazole

- Variety of mechanisms may lead to reduced azole susceptibility, including
  - Mutation at the azole target ErgII
  - Up-regulation of drug efflux pumps

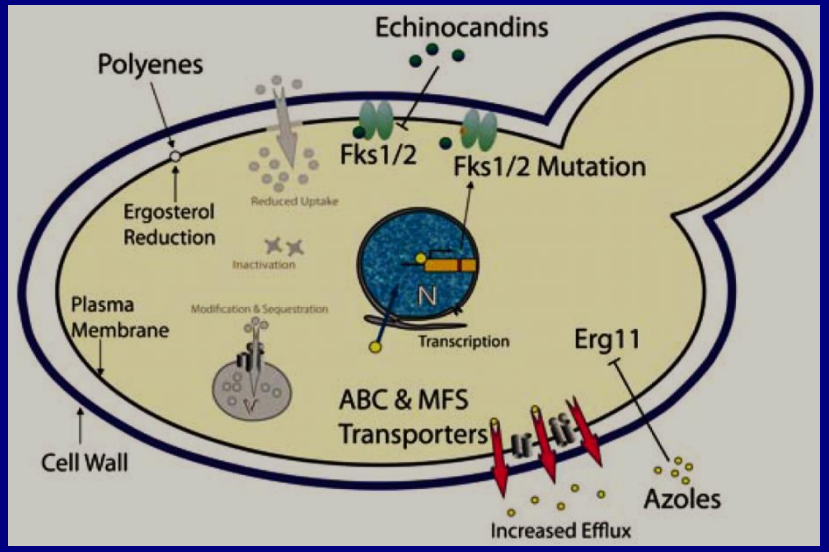


Image from Tscherner et al 2011
http://www.mdpi.com/1424-8247/4/1/169/htm

Antifungal Resistance
Modifications of the Ergosteral Pathway

- ERG 11 (ERG 16) - encodes 14-α LDM

- ERG 3 - encodes C-5 sterol desaturase
- Point mutations
  - T315A
  - R467K
  - R287K
- Overexpression
- Gene amplification (leads to overexpression)

Molecular Mechanisms of Azole Resistance Efflux

1. CDR proteins are ABC transporters (*Candida* drug resistance)

   - Found in wide variety of organisms and serve as efflux pumps to most azoles

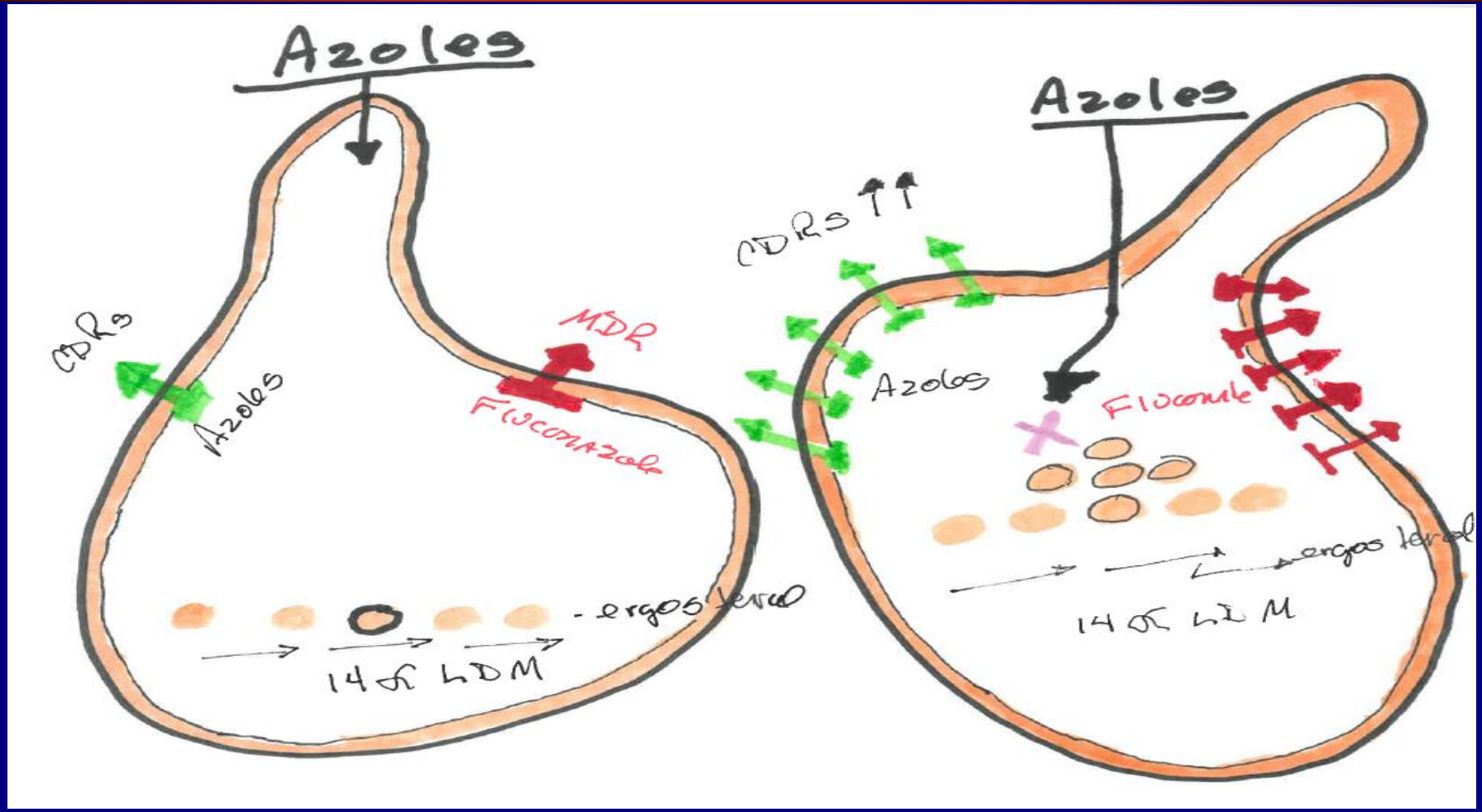
   - Genes encode for transmembrane pores, cassettes and metabolic energy for pump

     - CDR 1- Flz/Itz
     - CDR 2 – azoles and terbinafine

2. MDR gene encodes for membrane pores which are specific for fluconazole (MDR 1- Flz resistnce)

Both CDR and MDR 1 genes are overexpressed in azole-resistant *C. albicans*

Azole Resistance



Courtesy of me

Echinocandin Resistance Issues

- What are the breakpoints for echinocandins ? (< 2 vs < 4 ??)
- Difficult to establish in vitro, but can happen:
  - Balashov et al AAC 2006;50:2058-63.
- Clinical resistant isolates at this point are uncommon, but they are increasing:
  - *C. albicans* from HIV-positive with rOPC/EC MIC > 64 µg/ml
  - *C. parapsilosis* PVE (resistant to Cfgn & Mfgn, but not Afgn)
  - *C. parapsilosis* burn unit ~ 20 isolates (all colonizers, no infections); also same resistance pattern.
  - *C. glabrata* clinical isolates (~ 5% 2001 to 12% in 2010)

Hernandez S, et al AAC 2004;48:1382-83; Balashov SV, et al AAC 2006;50:2058-63.

Moudgal V, et al AAC 2005;49:767-69.

Candida spp. Echinocandin Resistance Mechanisms

- *Very little is known – probably several mechanisms:*
  - Substitutions in the Fks1p gene (Asn 470 with Lys, Leu642 with Ser, and Ser645 substitutions)
    - (Ohyama T, et al. AAC 48;319:2004;Park S, et al. AAC 49;3264:2005)
  - Mutations in the FEN1 gene involved in sphingolipid biosynthesis
    - (El-sherbeini et al. J Bact 177:3227:1995)
  - Mutations in the CKA2 gene encoding a protein kinase
    - (Edlind T, personal communication, 2005)
  - Phytosphingosine (PHS) accumulation
    - (cka2 protein kinase mutants are deficient in ceramide synthase are accumulate the ceramide precursor PHS)
    - Katiyar S, Edlind T. ASM Candiasis Meeting 2004, abstract S8:3)

C. parapsilosis in PVE MICs (µg/ml)

Isolate	FLz	Vcz	Csp	Mfgn	Afgn	AmB
1	1	0.03	2	8	1	0.25
2	➢64	➢16	➢16	➢16	2	0.5
3	➢64	➢16	➢16	➢16	2	0.5
6	➢64	➢16	➢16	➢16	2	0.5

Moudgal V, et al AAC 2005;49:767-69.

Case 2

55 year old male attorney underwent Colectomy for Dukes A colon CA

Day 2 post op:	Antibiotics started for suspected post-op wound infection
Day 6:	New fever B.C. drawn
Day 8:	B.C. (+) for *C. glabrata* in Bactec bottles 1/4
	Central venous catheter removed
	Afebrile after 24 hours
	Initiate antifungal therapy with:

Case 2

What is the preferred agent to initiate antifungal therapy for a *C. glabrata bloodstream infection*:

A. Fluconazole 400 mg daily
B. Fluconazole 800 mg daily
C. Liposomal amphotericin B 3mg/kg daily
D. Echinocandin daily
E. Voriconazole 6 mg/kg q 12 hrs IV

Case 2

What is the preferred agent to initiate antifungal therapy for a *C. glabrata bloodstream infection*:

A. Fluconazole 400 mg daily
B. Fluconazole 800 mg daily
C. Liposomal amphotericin B 3mg/kg daily
D. Echinocandin daily – correct
E. Voriconazole 6 mg/kg q 12 hrs IV

Current IDSA/MSG recommendations include initiating therapy with an echinocandin until spcies and MICs are known.

Pappas PG et al CID Dec 16th 2015

Case 2

So what about if the MIC to fluconazole comes back 4 days later as 2 mcg/ml?

Can you de-escalate to fluconazole?

* yes or no

What dose:

A. Fluconazole 400 mg daily
B. Fluconazole 800mg daily

Case 2

So what about if the MIC to fluconazole comes back 4 days later as 2 mcg/ml?

Can you de-escalate to fluconazole?

* yes or no

What dose:

A. Fluconazole 400 mg daily - MIC 2 is susceptible at dose of 400mg daily or 6mg/kg daily
B. Fluconazole 800mg daily - no need for such a dose, although many individuals will use this dose for all *C. glabrata*.

Pappas PG et al CID Dec 16th 2015

Aspergilosis

- Ubiquitous (>600 species)
  - Sources:
    - Soil, organic debris
    - Hospitals (ventilation system, dust, water supply)
- Portals of Entry
  - Inhalation of conidia - ~90%
  - Traumatized skin
  - Traumatized gastrointestinal lining

Aspergillus spp. Major Etiologic Agents

Aspergillus fumigatus	~60%
Aspergillus flavus	~ 15%
Aspergillus niger	~ 7%
Aspergillus terreus	~ 4% (intrinsic AmB-R)
Aspergillus glaucus	~ 1%
Aspergillus nidulans	~ 1%
Aspergillus ustus	< 1% (intrinsic AmB-R)

**A nidulans* (n=1), *A versicolor* (n=2), *A oryzae* (n=1), *A glaucus* (n=1).
Adapted from Patterson TF et al, 2000.

Aspergillus and Resistance

- Remains uncommon but is increasing in frequency, especially in the EU
- In vivo emergence of resistance is very rare and can occur during extended azole therapy in compromised host
- MDR to azoles only
- Ex vivo evolution of resistance from environmental *Aspergillus* spp. associated with plants (plant bulbs)
- Specifically due to fungicides which alter sterols (14$^{\alpha}$-demethylation
  - Alteration in cyp51A point mutations & gene overexpression

* ***A nidulans*** **(n=1),** ***A versicolor*** **(n=2),** ***A oryzae*** **(n=1),** ***A glaucus*** **(n=1).**
Adapted from Patterson TF et al, 2000.

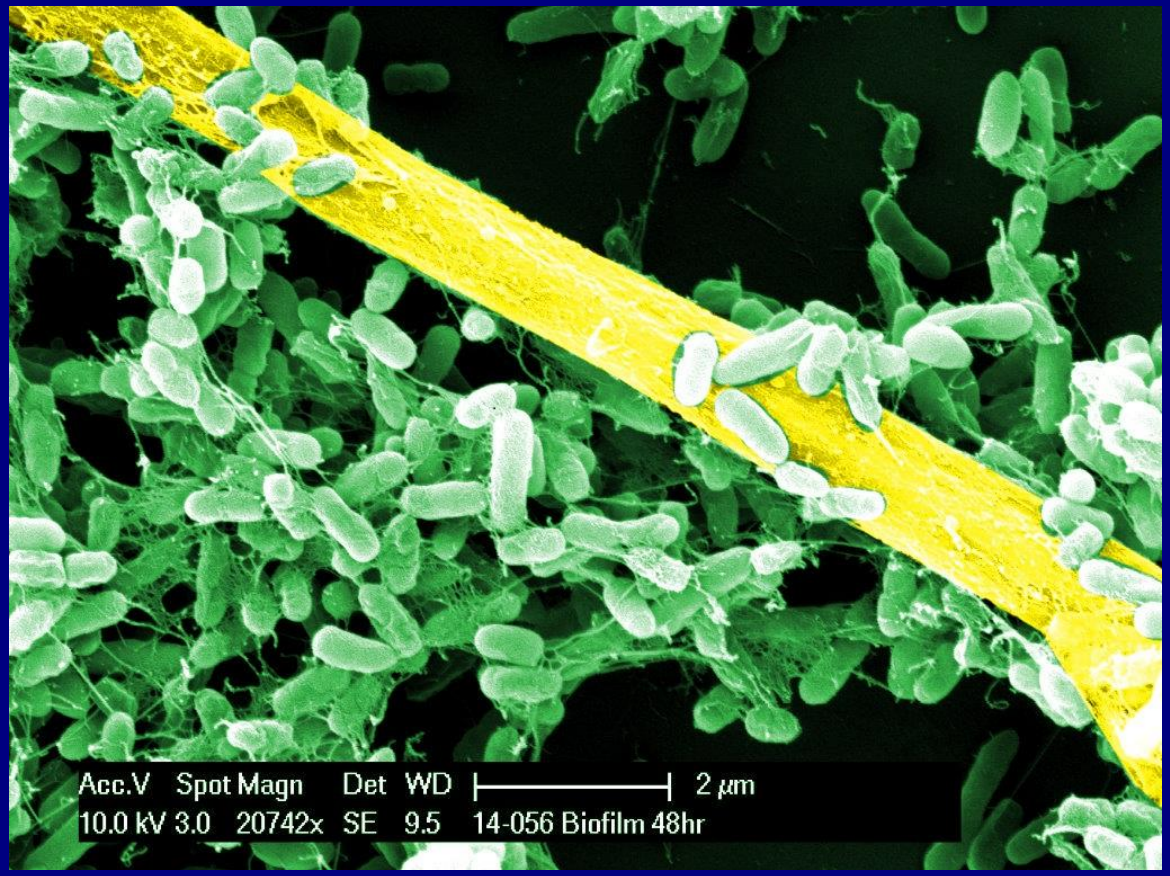
The Fungal Burden of Food

Pepper	100%	A. fumigatus	3+
	100%	A. flavus	3+
	100%	Mucorales	3+
Tea	100%	A. fumigatus	3+
	100%	A. niger	3+
	33%	Mucorales	3+
Freeze dried soup	20%	A. fumigatus/flavus	2+
Sweet biscuits	15%	Mucorales	+
Soft cheese	100%	Geotrichum	3+
Marijuana	100%	Aspergillus spp.	3+

Clinical/Financial Impact of Biofilms

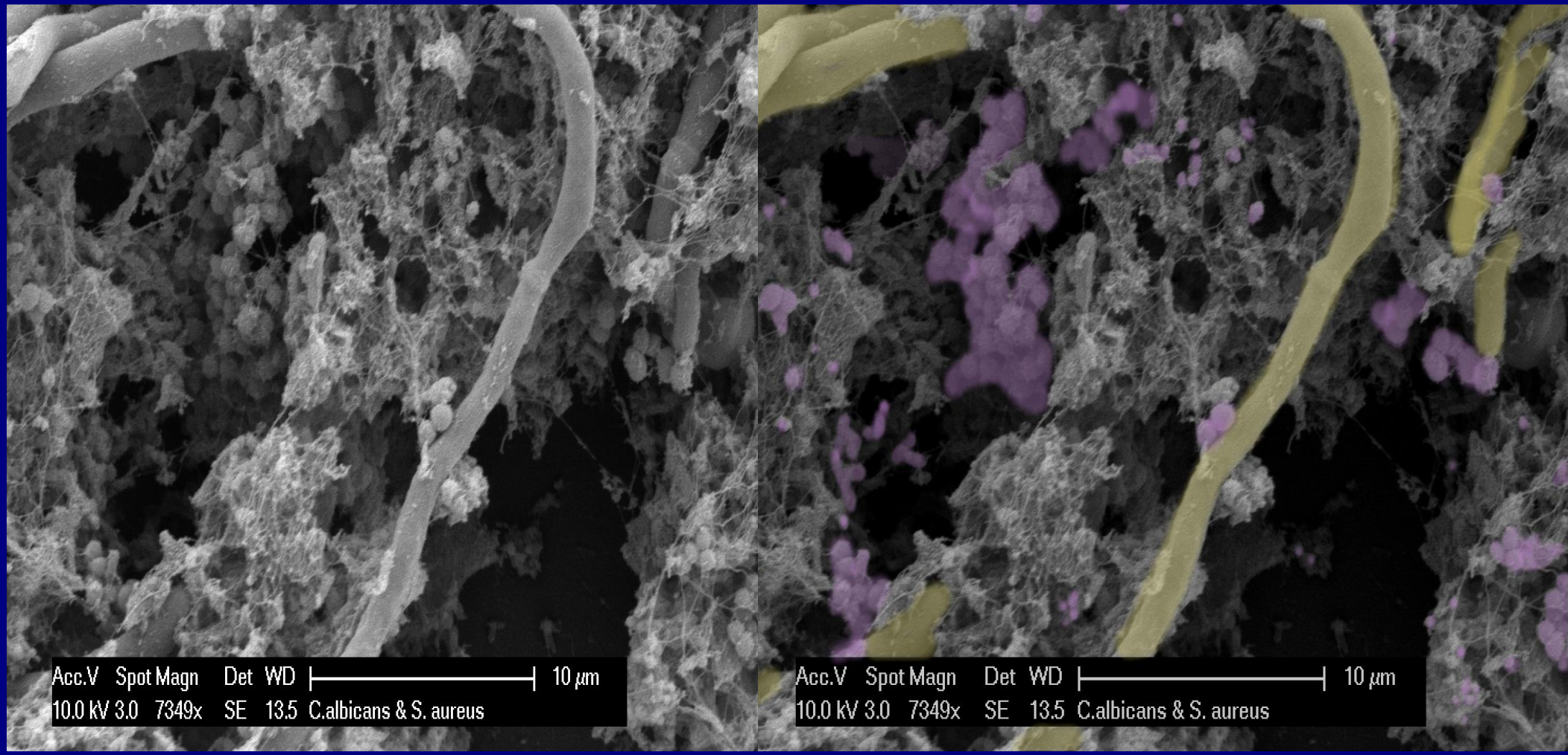
- Percival et al, WOUNDs, 2004…
  - Biofilms are associated with 65% of nosocomial infections.
  - The costs associated with the treatment of these biofilm associated infections exceeds $1 billion/year in the U.S.
  - Associated with increased resistance
- NIH press release, 2006
  - Biofilms are responsible for about 1 million medical device-related infections/year and biofilms are believed to be responsible for about 65% of chronic infections.

Polymicrobial Biofilm
Aspergillus-P. aeruginosa



Courtesy of me

Polymicrobial Biofilm
Candida albicans-S. aureus



Courtesy of me

Polyene Resistance

- Uncommon but not rare
  - 1º resistance > 2º resistance
- Primary resistance
  - *C. lusitaniae*
  - *G. guillermondii*
- Primary tolerance (Higher MICs)
  - *C. krusei*
  - *C. glabrata*
- Secondary resistance - Rare
  - *C. parapsilosis, C. tropicalis*

Future Antifungals

- Fosmanogepix (Amplyx-Pfizer)
  - Prodrug that targets Gwt1 enzyme that catalyzes early step of GPI (glycosylphosphatidylinositol-anchored pathway
  - Broad spectrum yeast and moulds
  - Effective against *C. auris* and MDR *Candida, Cryptococcus, Aspergillus, Fusarium*
- Olorofilm (F2G)
  - dihydroorotate dehydrogenase (DHODH) enzyme (pyrimidine synthesis)
  - *Broad spectrum also Aspergillus, Fusarium, Scedosporium, Histo-Blast-Cocci*
  - *No yeast activity*

Mota Fernandes C, et al. AAC Feb 2021.

Differential Diagnosis
Extra credit???

Courtesy of J Vazquez

Dead Mans Finger's

Xylaria polymorpha

* found on or near stumps of dead trees

Courtesy of J Vazquez

Conclusion

- Echinocandins have emerged as preferred initial therapy for most episodes of candidemia & invasive candidiasis
  - Based on:
    - Safety profile
    - Early fungicidal activity
    - Trend toward better outcomes
    - Increased rates of azole-resistant *Candida* species
- Gradual increase echinocandin resistance over past decade
- Emergence of multidrug-resistant *Candida* species is a growing concern

Questions?