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PHRI

Antifungal resistance among *Candida* spp

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Disclosures

D.S.P. receives funding from US National Institutes of Health and contracts from the CDC, Astellas, Scynexis, Cidara and Amplyx. He serves on advisory boards for Astellas, Cidara, Amplyx, Scynexis, and Matinas. In addition, D.S.P. has an issued US patent concerning echinocandin resistance and patents for the detection antifungal drug resistance.

Why Patients Fail Antimicrobial Therapy

- **Clinical response is a blend of drug, host and microbial factors**

Non-microbiological

- ✓ Unfavorable PK
- ✓ Poor drug penetration
- ✓ Co-morbidities
- ✓ Host reservoirs
  - GI tract; intra-abdominal abscesses

Microbiological

- ✓ Drug resistance
  - Inherent
  - Acquired

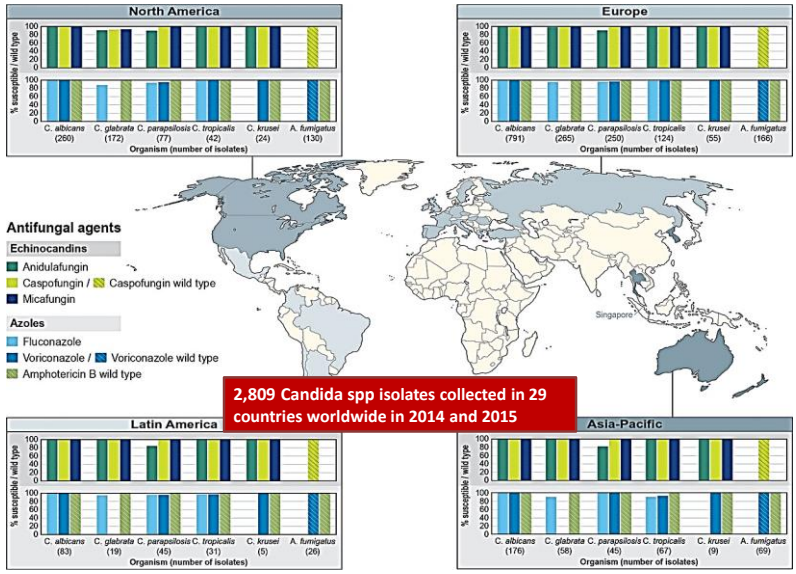
ANTIFUNGAL DRUG RESISTANCE

Resistance is best defined as clinical failure following drug therapy due to organisms that are refractory to drugs at a therapeutic dosage.

Resistance occurs with all antifungal agents, but resistance to triazole and echinocandin drugs is the most clinically significant, and strains resistant to multiple drug classes have emerged

What are the current trends and underlying genetic mechanisms that confer acquired drug resistance?

Global view of echinocandin and azole susceptibility (ECVs) among *Candida*



Susceptibility remains high among most *Candida* species

Castanheira et al. 2017 AAC

Antifungal activity using CLSI reference method against invasive yeasts collected in 29 countries worldwide in 2014 and 2015

Organism (no. of isolates tested) and antifungal agent	MIC_{50} or MEC_{50} (µg/ml)	MIC_{90} or MEC_{90} (µg/ml)	% of isolates			
			CLSI method		ECV	
			S	R	WT	NWT
C. albicans (1,310)						
Anidulafungin	0.015	0.03	99.9	0.0	99.8	0.2
Caspofungin	0.015	0.03	99.8	0.2		
Micafungin	0.015	0.03	99.8	0.2	99.8	0.2
Fluconazole	≤0.12	0.25	99.6	0.4		
Voriconazole	≤0.008	0.015	99.9	<0.1		
Posaconazole	0.03	0.06				
Amphotericin B	1	1			100.0	0.0
C. glabrata (514)						
Anidulafungin	0.06	0.12	95.9	2.5	97.5	2.5
Caspofungin	0.03	0.06	96.9	2.5		
Micafungin	0.015	0.03	97.5	2.3	96.9	3.1
Fluconazole	4	16	92.0	8.0		
Voriconazole	0.12	0.5				
Posaconazole	0.5	1				
Amphotericin B	1	1			100.0	0.0
C. parapsilosis (417)						
Anidulafungin	2	4	88.7	0.0	100.0	0.0
Caspofungin	0.25	0.5	100.0	0.0		
Micafungin	1	2	100.0	0.0	100.0	0.0
Fluconazole	0.5	2	95.7	3.8		
Voriconazole	0.015	0.03	96.4	0.7		
Posaconazole	0.06	0.12				
Amphotericin B	1	1			100.0	0.0
C. tropicalis (264)						
Anidulafungin	0.015	0.03	100.0	0.0	100.0	0.0
Caspofungin	0.015	0.06	100.0	0.0		
Micafungin	0.015	0.06	100.0	0.0	99.6	0.4
Fluconazole	0.25	0.5	96.2	2.7	96.2	3.8
Voriconazole	0.015	0.06	97.0	2.3		
Posaconazole	0.03	0.12				
Amphotericin B						

Resistance among *Candida* species is low

Castanheira et al. 2017 AAC

Int J Antimicrob Agents. 2011 Jul;38(1):65-9.

Candida bloodstream infections: comparison of species distribution and resistance to echinocandin and azole antifungal agents in Intensive Care Unit (ICU) and non-ICU settings in the SENTRY Antimicrobial Surveillance Program (2008-2009).

Pfaller MA[1], Messer SA, Moet GJ, Jones RN, Castanheira M.

Abstract

Minimum inhibitory concentration (MIC) data from the SENTRY Antimicrobial Surveillance Program generated by reference methods were analysed to compare the antifungal resistance profiles and species distribution of Candida bloodstream infection (BSI) isolates obtained from patients in the Intensive Care Unit (ICU) and those from non-ICU locations. Results from

79 medical centres between 2008 and 2009 were tabulated. MIC values were obtained for anidulafungin, caspofungin, micafungin, fluconazole, posaconazole and voriconazole. Recently revised Clinical and Laboratory Standards Institute breakpoints for resistance were employed. **A total of 1752 isolates of Candida spp. were obtained from ICU (779; 44.5%) and non-ICU (973; 55.5%) settings.** The frequency of ICU-associated Candida BSI was higher in Latin America (56.5%) compared with Europe (44.4%) and North America (39.6%). The frequency of candidaemia in the ICU decreased both in Latin America and North America over the 2-year study period. Approximately 96% of isolates both in ICU and non-ICU settings were caused by only five species (Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis and Candida krusei). Resistance both to azoles and echinocandins was uncommon in ICU and non-ICU settings. **Overall, fluconazole resistance was detected in 5.0% of ICU isolates and 4.4% of non-ICU isolates. *Candida glabrata* was the only species in which resistance to azoles and echinocandins was noted, and this multidrug-resistant phenotype was found in both settings.** In conclusion, the findings from this global survey indicate that invasive candidiasis can no longer be considered to be just an ICU-related infection, and efforts to design preventive and diagnostic strategies must be expanded to include other at-risk populations and hospital environments. Concern regarding C. glabrata must now include resistance to echinocandins as well as azole antifungal agents

Prevalence comparable to that reported by SENTRY Antimicrobial Surveillance Program for 2008-09

INDIA

Antimicrob Agents Chemother. 2018 May 25;62(6).

Absence of Azole or Echinocandin Resistance in Candida glabrata Isolates in India despite Background Prevalence of Strains with Defects in the DNA Mismatch Repair Pathway.

Singh A[1], Healey KR[2], Yadav P[1], Upadhyaya G[1], Sachdeva N[3], Sarma S[4], Kumar A[5], Tarai B[6], Perlin DS[2], Chowdhary A[7].

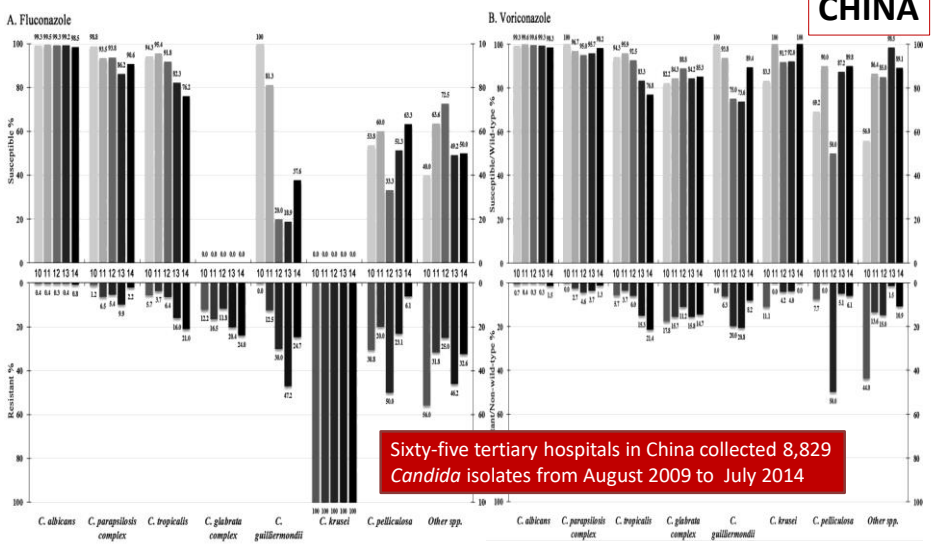
Abstract

Candida glabrata infections are increasing worldwide and exhibit greater rates of antifungal resistance than those with other species. DNA mismatch repair (MMR) gene deletions, such as *msh2Δ*, in *C. glabrata* resulting in a mutator phenotype have recently been reported to facilitate rapid acquisition of antifungal resistance. **This study determined the antifungal susceptibility profiles of 210 *C. glabrata* isolates in 10 hospitals in India** and investigated the impact of novel *MSH2* polymorphisms on mutation potential. **No echinocandin- or azole-resistant strains and no mutations in *FKS* hot spot regions were detected among the *C. glabrata* isolates, supporting our *in vitro* susceptibility testing results.** CLSI antifungal susceptibility data showed that the MICs of anidulafungin (geometric mean [GM], 0.12 μg/ml) and micafungin (GM, 0.01 μg/ml) were lower and below the susceptibility breakpoint compared to that of caspofungin (CAS) (GM, 1.31 μg/ml). Interestingly, 69% of the *C. glabrata* strains sequenced contained six nonsynonymous mutations in *MSH2*, i.e., V239L and the novel mutations E459K, R847C, Q386K, T772S, and V239/D946E. Functional analysis of *MSH2* mutations revealed that 49% of the tested strains (40/81) contained a partial loss-of-function *MSH2* mutation. The novel *MSH2* substitution Q386K produced higher frequencies of CAS-resistant colonies upon expression in the *msh2Δ* mutant. However, expression of two other novel *MSH2* alleles, i.e., E459K or R847C, did not confer selection of resistant colonies, confirming that not all mutations in the *MSH2* MMR pathway affect its function or generate a phenotype of resistance to antifungal drugs. The lack of drug resistance prevented any correlations from being drawn with respect to *MSH2* genotype.

All *C. glabrata* isolates were fully susceptible—no drug resistance detected

Trends of fluconazole (A) and voriconazole (B) susceptibility and resistance rates of Candida species determined through the CHIF-NET study (2010 to 2014).

CHINA



Sixty-five tertiary hospitals in China collected 8,829 *Candida* isolates from August 2009 to July 2014

But not in China where significant azole resistance detected among certain *Candida* spp.

Meng Xiao et al. J. Clin. Microbiol. 2018;56:e00577-18

DENMARK

J Clin Microbiol. 2018 Mar 26;56(4).

Update from a 12-Year Nationwide Fungemia Surveillance: Increasing Intrinsic and Acquired Resistance Causes Concern.

Astvad KMT, Johansen HK, Røder BL, Rosenvinge FS, Knudsen JD, Lemming L, Schønheyder HC, Hare RK, Kristensen L, Nielsen L, Gertsen JB, Dzajic E, Pedersen M, Østergård C, Olesen B, Søndergaard TS, Arendrup MC

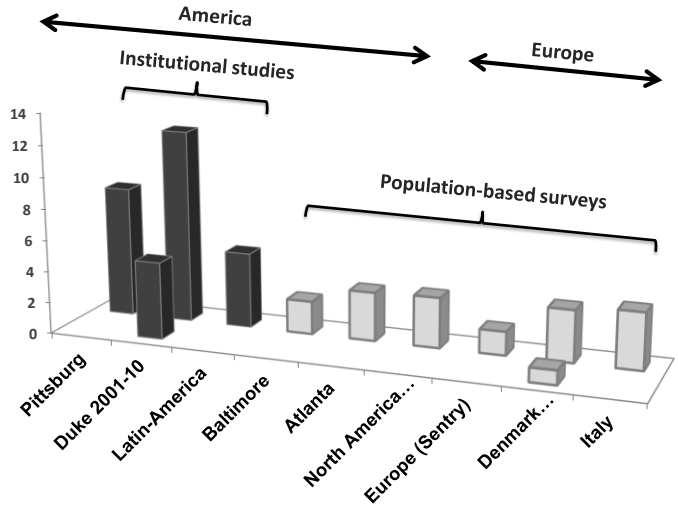
Abstract

New data from the years 2012 to 2015 from the Danish National Fungemia Surveillance are reported, and epidemiological trends are investigated in a 12-year perspective (2004 to 2015). During 2012 to 2015, 1,900 of 1,939 (98%) fungal bloodstream isolates were included. The average incidence was 8.4/100,000 inhabitants, and this appears to represent a stabilizing trend after the increase to 10.1/100,000 in 2011. The incidence was higher in males than females (10.0 versus 6.8) and in patients above 50 years, and those changes were mainly driven by an increasing incidence among 80-to-89-year-old males (65.3/100,000 in 2014 to 2015). The proportion of *Candida albicans* isolates decreased from 2004 to 2015 (64.4% to 42.4%) in parallel with a doubling of the proportion of *Candida glabrata* isolates (16.5% to 34.6%, $P < 0.0001$). *C. glabrata* was more common among females (34.0% versus 30.4% in males). Following an increase in 2004 to 2011, the annual drug use stabilized during the last 2 to 3 years of that time period but remained higher than in other Nordic countries. This was particularly true for the fluconazole and itraconazole use in the primary health care sector, which exceeded the combined national levels of use of these compounds in each of the other Nordic countries.

Fluconazole susceptibility decreased (68.5%, 65.2%, and 60.6% in 2004 to 2007, 2008 to 2011, and 2012 to 2015, respectively, $P < 0.0001$), and echinocandin resistance emerged in *Candida* (0%, 0.6%, and 1.7%, respectively, $P < 0.001$). Amphotericin B susceptibility remained high (98.7%). Among 16 (2.7%) echinocandin-resistant *C. glabrata* isolates (2012 to 2015), 13 harbored FKS mutations and 5 (31%) were multidrug resistant. The epidemiological changes and the increased incidence of intrinsic and acquired resistance emphasize the importance of continued surveillance and of strengthened focus on antifungal stewardship.

In Denmark, resistance is a growing problem especially among *C. glabrata*

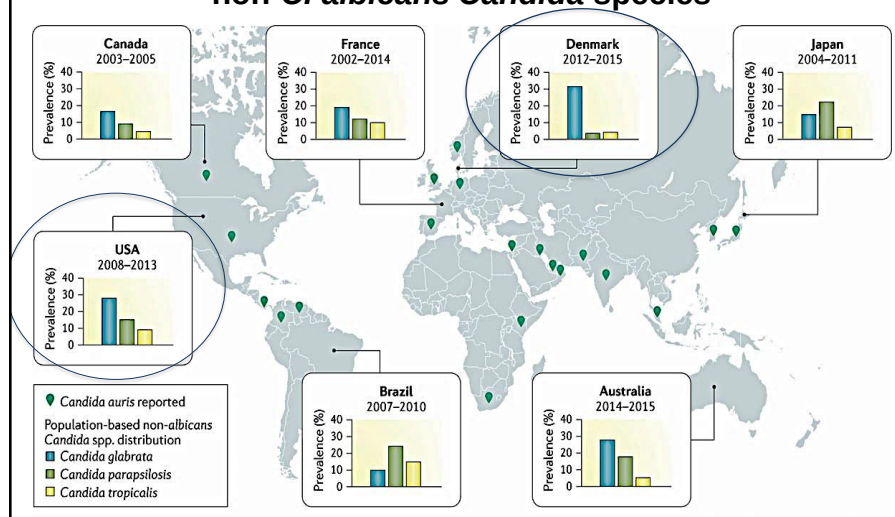
Echinocandin resistance in *C. glabrata*



Resistance rate varies among different studies. The rate reported from institutional studies are higher than that from population-based surveys

Perlin, Shor and Zhao 2015 CCMR

Geographical variations in the distribution of non-*C. albicans Candida* species



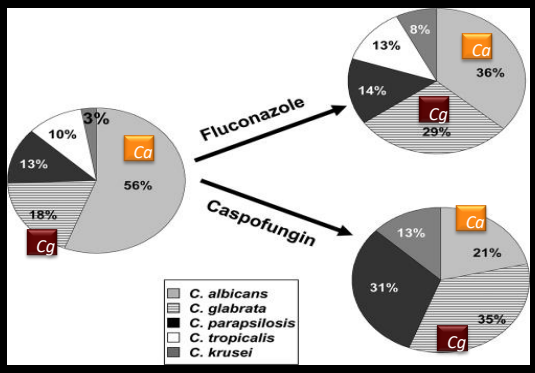
Data presented are from Australia, Brazil, Canada, Denmark, France, Japan and the United States6,[200–205]. Data on *C. auris* are from the Centers for Disease Control and Prevention (last accessed 27 March 2018).

Nature Reviews | Disease Primers

Higher prevalence correlates with greater resistance

Pappas, P. G. et al. (2018) Invasive candidiasis *Nat. Rev. Dis. Primers*

Prior azole and echinocandin exposure is shifting the distribution of *Candida* spp. recovered from severely ill patients with *C. glabrata* as a major cause of disease.



Proportion of the five major *Candida* species responsible for fungemia in patients with (159) or without (2,289) prior exposure to FLZ or with (61) or without (2,387) prior exposure to CSF

Lortholary et al. 2011 AAC

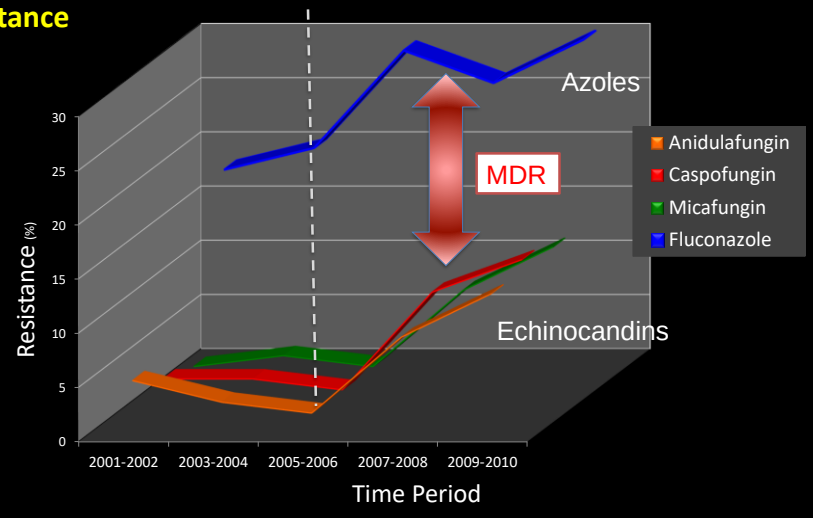
C. glabrata has emerged as the dominant pathogen in certain immunocompromised patient populations

Table 1. Distribution of Different *Candida* Species Among Patients With Hematologic Malignancies and Solid Tumors

Candida species	No. of patients (%) HM n = 281	ST n = 354	*P*
C. albicans	38 (14)	161 (45)	<.001
C. glabrata	86 (31)	64 (18)	<.001
C. krusei	68 (24)	6 (2)	<.001
C. parapsilosis	39 (14)	71 (20)	.045
C. tropicalis	27 (10)	30 (9)	.620
C. guilliermondii	4 (1)	2 (1)	.410
C. lusitaniae	3 (1)	3 (1)	.990
Mixed *Candida* species	16 (6)	17 (5)	.620

Hachem et al. 2008 Cancer

Concomitant rise in azole and echinocandin resistance among *Candida glabrata* isolates with emergence of multidrug (MDR) resistance



Alexander et al 2013 CID

MDR increasingly more common

146 Total Isolates

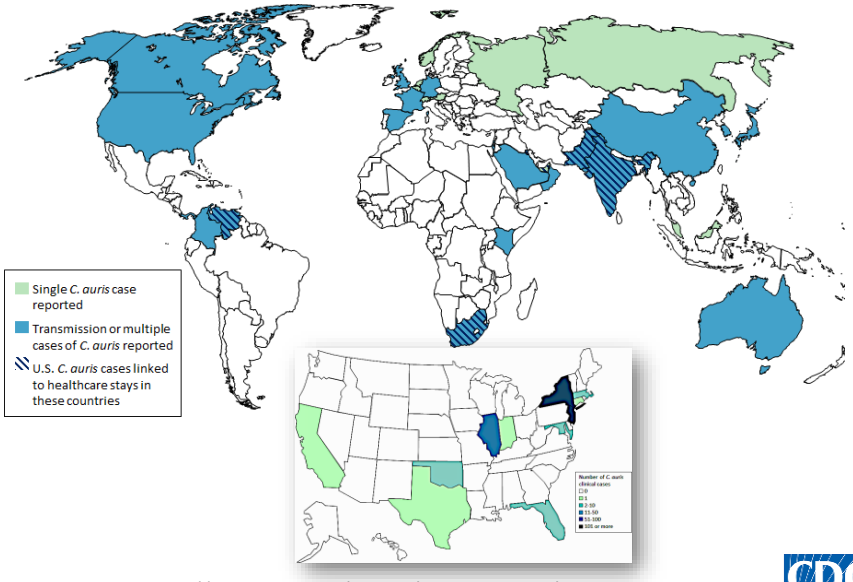
- 20.5% FLU^r
- 10.3% CSF^r
- 6.8% MDR (CSF, FLU, AMB) MDR

***C. glabrata*–positive blood cultures at MD Anderson Cancer Center, Houston, TX during March 2005–September 2013**

Resistance to echinocandins, azoles and polyenes

Farmakiotis et al. EID. 2014

Global and regional maps depicting rapid emergence of multidrug-resistant clinical *Candida auris* strains



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

Microbiologyopen. 2018 Aug; 7(4): e00578
Candida auris: A **systematic review** and meta-analysis of **current updates** on an **emerging** multidrug-resistant **pathogen**
John Osei Sekyere

Antifungal resistance profiles of the estimated 742 *C. auris* isolates

Most of the isolates were resistant to:

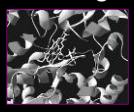
- **Fluconazole (*n* ≥ 318; 44.29%)**
- **Voriconazole (VRZ) (*n* ≥ 91; 12.67%),**
- **Itraconazole (ITZ) (*n* ≥ 13; 1.81%)**
- **Isavuconazole (ISA) (*n* ≥ 11; 1.53%),**
- **Posaconazole (PSZ) (*n* ≥ 10; 1.39%)**
- **AMB (*n* ≥ 111; 15.46%)**
- **Flucytosine (FCN) (*n* ≥ 14; 1.95%)**
- **Caspofungin (*n* ≥ 25; 3.48%),**
- **Anidulafungin (*n* ≥ 9; 1.25%)**
- **Micafungin (*n* ≥ 9; 1.25%)**
- **SCY-078 (0; 0%)**

Resistance to at least two of these drugs were frequently reported

What are the mechanisms of azole and echinocandin resistance among *Candida* species?

Azole resistance mechanisms

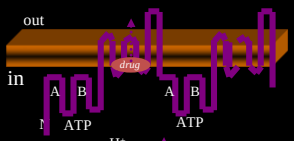
I. **Alteration of Drug Target**



Erg11 Candida spp.
Erg5 Candida spp.
Erg11 Cr. neoformans
Cyp51A Aspergillus spp.

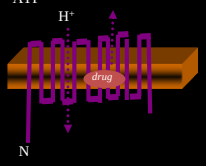
II. **Over-expression of Efflux Transporters**

ABC



Cdr1, C. albicans
Cdr1 C. glabrata
Pdh1 C. glabrata
Mdr1,2,4 A. fumigatus
Afr1 C. neoformans

MFS



Mdr1 C. albicans
Flu1 C. albicans
Mcm1 C. albicans
Mdr3 A. fumigatus

III. **Over-expression of Target Site**

Erg11 Candida spp.
Cyp51A A. fumigatus

IV. **Chromosomal abnormalities**
Aneuploidy, disomy, Isochromosomes
C. albicans, Cr. Neoformans
e.g. ERG11, TAC1

V. **Transcription factors**
TAC1, MRR1, PDR1
UPC2

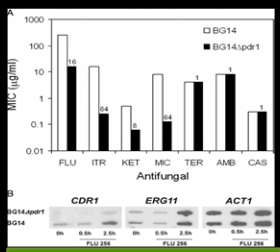
V. **Heteroresistance**
(Genetic and epigenetic)
C. glabrata, Cr. neoformans, C. tropicalis

Azole resistance in *Candida glabrata*: a perfect storm for multidrug resistance

GOF Mutations in TF Pdr1

Azole resistance

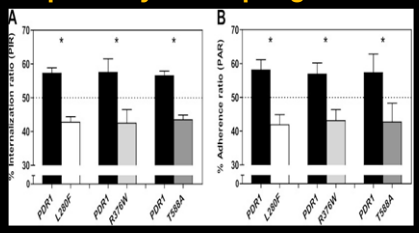
Upregulation of CDR1



Vermitsky et al. 2006 MM

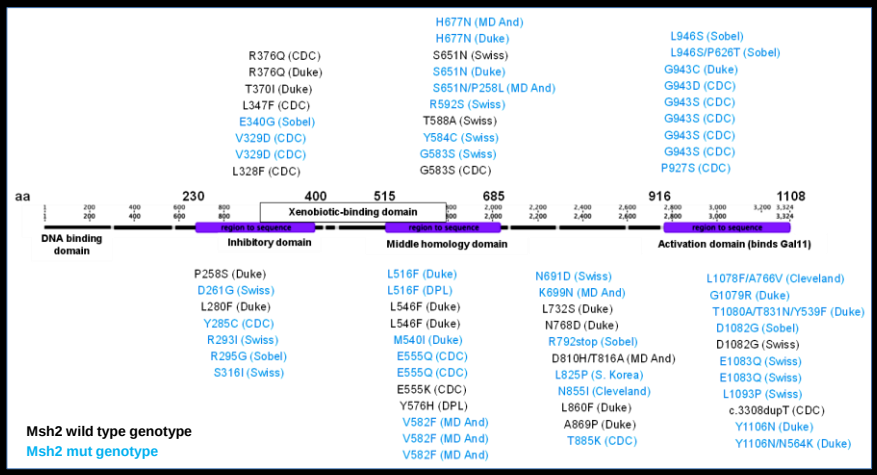
Increased burden

Decreased adherence and uptake by macrophages



Vale-Silva et al. 2013 Infect Immun

pdr1 GOF mutations identified within fluconazole-resistant clinical isolates



Healey, unpubl

Target Site amino acid substitutions in Erg11 account for AZOLE Resistance in *C. auris*

India (n=40)

Erg11	Prevalence	Elevated MICs
WT	2.5% (n=1)	- -
Y132F	55% (n=22)	FLC, VRC, IVC
K143R	42.5% (n=17)	FLC

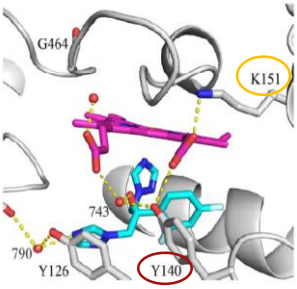
Colombia (n=56)

WT	62.5% (n=35)	WT
Y132F	3.5% (n=2)	FLC, VRC, IVC
K143R	1.8% (n=1)	FLC
I466M	30.4% (n=17)	FLC
Y501H	1.8% (n=1)	FLC, VRC, IVC

Kordalewska 2018

Mutations in Erg11 confer resistance in *S. cerevisiae* tester strain

- Expression of *C. auris erg11 mutations* in *S. cerevisiae*
  - *Saccharomyces cerevisiae* strain -BY4741 (haploid strain)
  - Plasmid - pRS416 (*S. cerevisiae* CEN/ARS; URA3; AMPr)
  - Amplified DNA fragment (*C. auris* ERG11) and linearized plasmid co-transformed into BY4741



Sagatova *et al.*, 2015 AAC

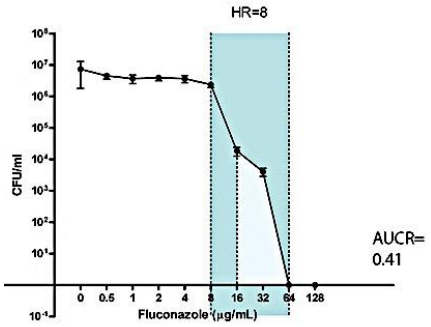
	FLC		VRC	
	48h	72h	48h	72h
BY4741 + empty	8	16	0.12	0.12
BY4741 + pCauErg11-WT	16	16	0.12	0.25
BY4741 + pCauErg11-Y132F	128	>128	2	4
BY4741 + pCauErg11-K143R	64	>128	0.5	1
BY4741 + pCauErg11-K177R/ N335S/E343D	8	16	0.12	0.25

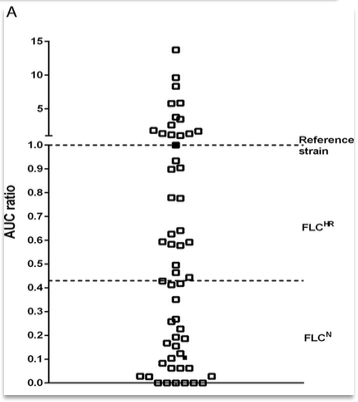
***C. auris* Y132 = *C. albicans* Y132 = *S. cerevisiae* Y140**
***C. auris* K143 = *C. albicans* K143 = *S. cerevisiae* K151**

HEALEY et al. AAC 2018

Heteroresistance refers to variability in the response to a drug within a clonal cell population.

Candida* and *Cryptococcus





- **Heteroresistant strains are not detected by standard susceptibility testing**
- **Heteroresistance to FLZ is a graded phenotype associated with ABC transporter upregulation and drug efflux- may reflect cellular and/or epigenetic adaptation**

R. Ben-Ami et al. 2016 mBio

Nat Commun. 2018 Jun 25;9(1):2470

Antifungal tolerance is a subpopulation effect distinct from resistance and is associated with persistent candidemia

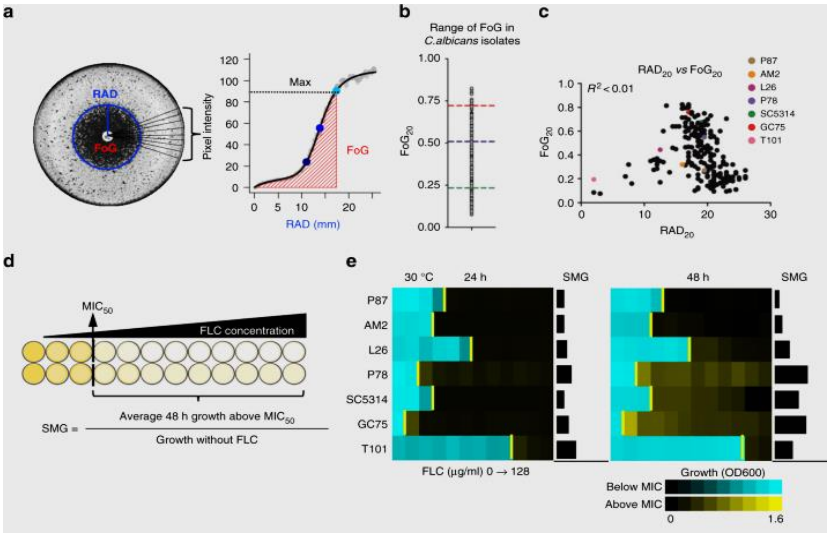
Alexander Rosenberg, Iuliana V. Ene, Maayan Bibi, Shiri Zakin, Ella Shtifman Segal, Naomi Ziv, Alon M. Dahan, Arnaldo Lopes Colombo, Richard J. Bennett & Judith Berman

Tolerance to antifungal drug concentrations above the minimal inhibitory concentration (MIC) is rarely quantified, and current clinical recommendations suggest it should be ignored. Here, we quantify antifungal tolerance in *Candida albicans* isolates as the fraction of growth above the MIC, and find that it is distinct from susceptibility/resistance.

Instead, tolerance is due to the slow growth of subpopulations of cells that overcome drug stress more efficiently than the rest of the population, and correlates inversely with intracellular drug accumulation. Many adjuvant drugs used in combination with fluconazole, a widely used fungistatic drug, reduce tolerance without affecting resistance. Accordingly, in an invertebrate infection model, adjuvant combination therapy is more effective than fluconazole in treating infections with highly tolerant isolates and does not affect infections with low tolerance isolates. Furthermore, isolates recovered from immunocompetent **patients with persistent candidemia display higher tolerance than isolates readily cleared by fluconazole. Thus, tolerance correlates with, and may help predict, patient responses to fluconazole therapy.**

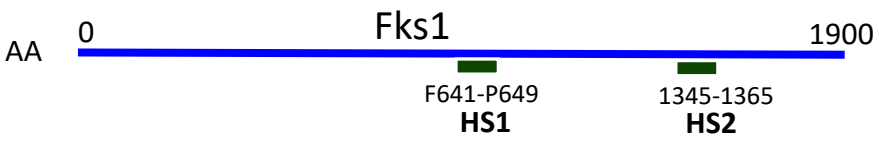
Fraction of growth (FoG) above fluconazole MIC may influence clinical response

Drug responses in disk diffusion assays (DDAs) and liquid broth microdilution assays (BMDAs). The fraction of growth inside the zone of inhibition (FoG) is the area under the curve (red) at the RAD threshold, divided by the maximum area.



Rosenberg et al. 2018 Nat Comm

Echinocandin resistance resulting in clinical failures involves mutations in two hot spot region of the Fks catalytic subunit of glucan synthase

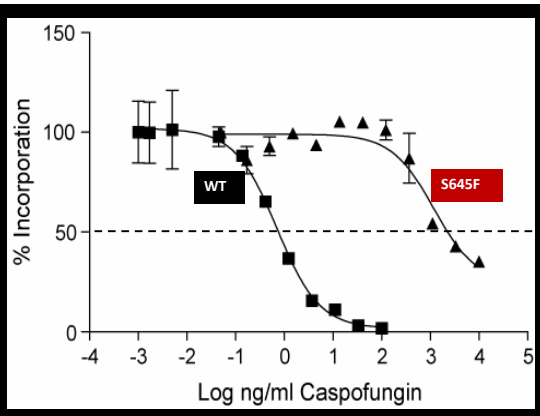


- **Two regions (HS1 and HS2) of Fks are associated with resistance in *Candida***
- **Prominent mutations confer cross-resistance to all echinocandin class drugs**
- **Azole resistance mechanisms are not active on echinocandin drugs**

Park et al. 2005 AAC

Altered drug-target interaction

Prominent HS1 mutation in *FKS1* decreases sensitivity of glucan synthase to echinocandin drugs by 100-10,000 fold



Kinetic inhibition of Glucan Synthase Activity-
UDP-D-[1-3] glucose Incorporation Assay

Park et al. 2005 AAC

Presence of an *FKS* mutation rather than MIC is the most significant risk factor for clinical failure

Performance of echinocandin breakpoints and *FKS* mutations in predicting therapeutic failure

Variable[a]	% sensitivity[b]	% specificity[c]	Likelihood ratio
CLSI breakpoints (intermediate isolates considered to be resistant[d])			
Caspofungin BP > 0.12 µg/ml	100 (10/10)	0 (0/29)	NA[e]
Anidulafungin BP > 0.12 µg/ml	30 (3/10)	97 (28/29)	8.7
Micafungin BP > 0.06 µg/ml	30 (3/10)	90 (26/29)	2.9
CLSI breakpoints (intermediate isolates considered to be sensitive[d])			
Caspofungin BP > 0.25 µg/ml	100 (10/10)	0 (0/29)	NA
Anidulafungin BP > 0.25 µg/ml	30 (3/10)	97 (28/29)	8.7
Micafungin BP > 0.12 µg/ml	20 (2/10)	97 (28/29)	5.8
Epidemiologic cutoff values			
Caspofungin BP > 0.12 µg/ml	100 (10/10)	0 (0/29)	NA
Anidulafungin BP > 0.25 µg/ml	30 (3/10)	97 (28/29)	8.7
Micafungin BP > 0.03 µg/ml	40 (4/10)	90 (26/29)	3.9
Cutoffs determined by ROC			
Caspofungin MIC > 0.5 µg/ml	60 (6/10)	86 (25/29)	4.3
Anidulafungin BP > 0.06 µg/ml	50 (5/10)	97 (28/29)	14.5
Micafungin BP > 0.03 µg/ml	40 (4/10)	90 (26/29)	3.9
FKS genotype			
Presence of mutation	60 (6/10)	97 (28/29)	17.4

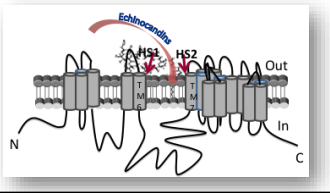
Shields et al. AAC 2012

Spectrum of Fks amino acid changes conferring clinical resistance- 17 year view

Organism	Fks1 Hot Spot1	Fks1 Hot Spot 2	Fks2 Hot Spot 1	Fks2 Hot Spot 2	Fks3 Hot Spot 1
C. albicans	F_{641}LTLSLRDP	DWIRRYTL	NO	NO	NO
C. krusei	F_{655}LILSIRDP	DWIRRYTL	NO	NO	NO
C. glabrata	F_{625}LILSIRDP	DWIRRYTL	F_{659}LILSLRDP	DWIRRYTL	NO
C. guilliermondii	F_{632}MALSIRDP	DWIRRYTL	NO	NO	NO
C. lypolytica	F_{662}LILSIRDP	DWIRRCVL	NO	NO	NO
C. tropicalis	F___LTLSIRDP	DWIRRYTL	NO	NO	NO
C. dubliniensis	F_{641}LTLSIRDP	DWIRRYTL	NO	NO	NO
C. auris	F_{635}LTLSLRDP	DWIRRYTL	NO	NO	NO
*C. parapsilosis**	F_{652}LTLSIRDA	DWIRRYTL	NO	NO	NO
C. orthopsilosis	F___LTLSIRDA	DWVRRYTL	NO	NO	NO
C. metapsilosis	F___LTLSIRDA	DWIRRYTL	NO	NO	NO

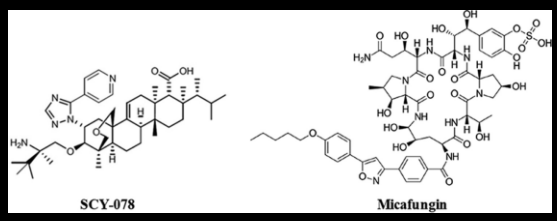
NO: Not Observed * *C. parapsilosis sensu* stricto ---: Incomplete sequence designation

Acquired Mutation Strong Resistance Acquired Mutation Weak Resistance Naturally- occurring polymorphism

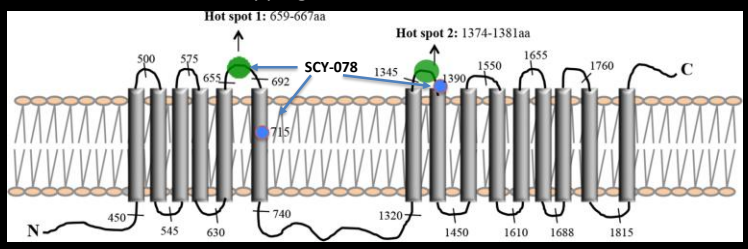


Perlin, 2015 MCM
Arendrup a and Perlin, OID 2014
Johnson and Edlind, 2012 Euk Cell

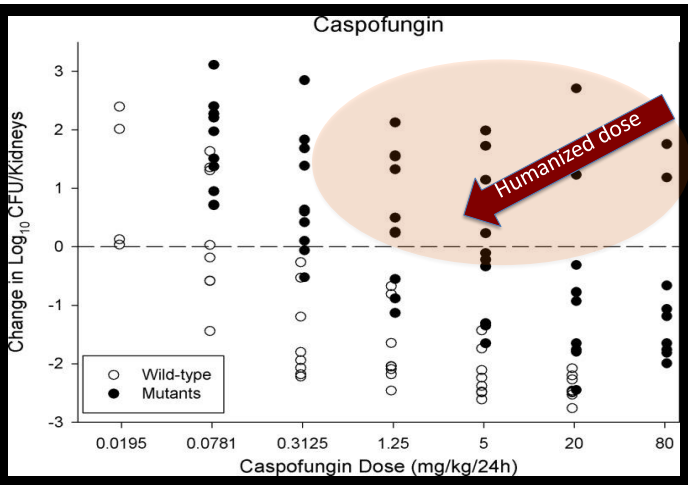
Resistance to glucan synthase inhibitor SCY-078, an enfumafungin, is mediated by amino acid changes within and near echinocandin hot spots



Overlapping but not identical



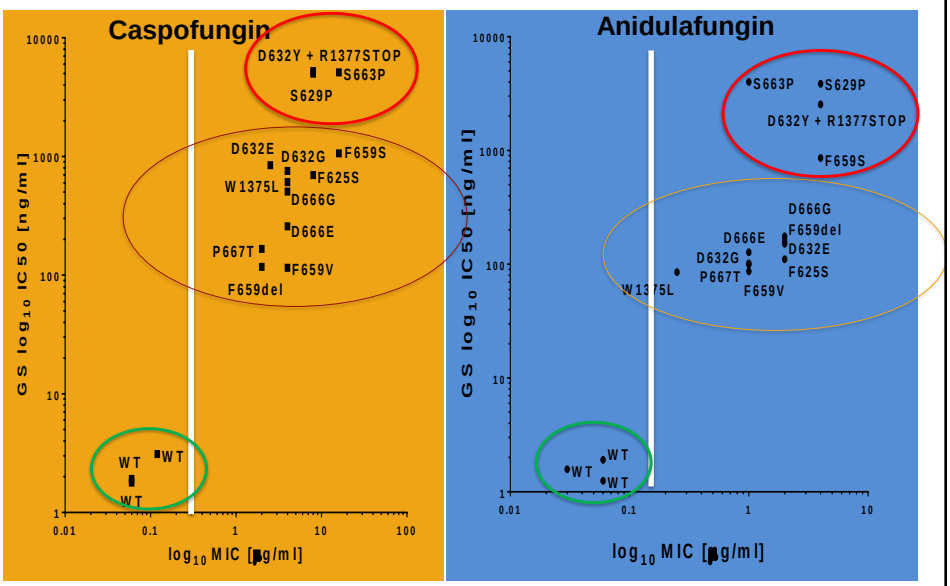
fks mutants are insensitive to high doses of CSF in a pharmacodynamic model of infection-FKS status matters



Dose-response relationships for caspofungin against multiple *C. glabrata* isolates, including wild type and *fks*-containing mutants.

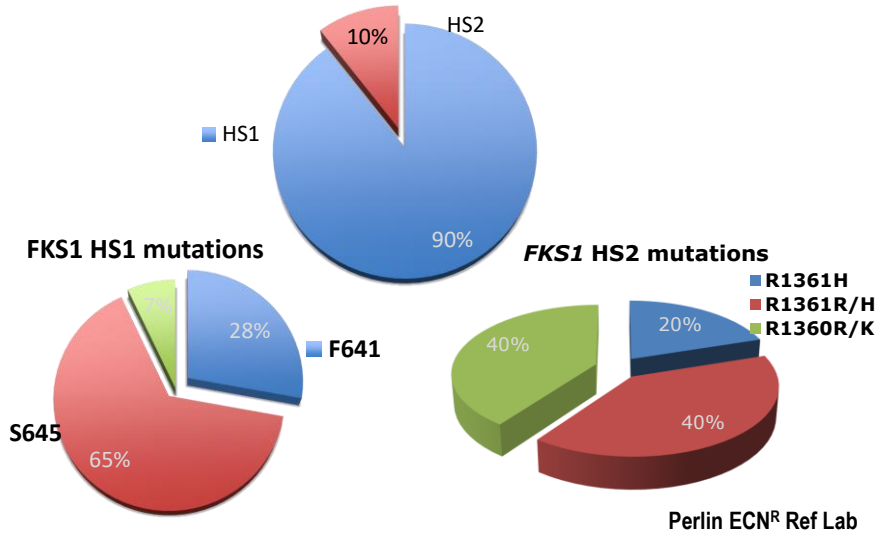
Lepak et al. 2012 AAC

Effect of FKS mutations on IC50 and MIC

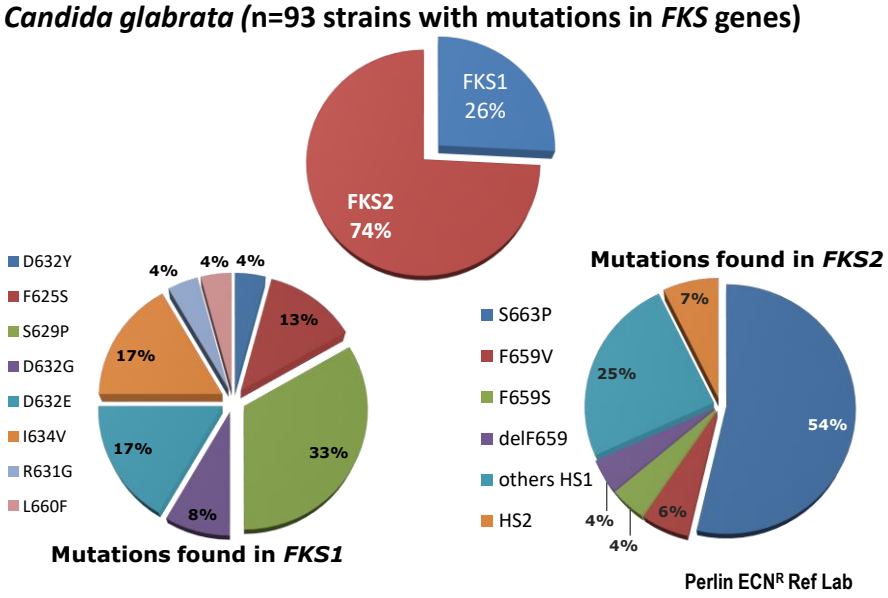


Astellas ECN Resistance Ref Center

In C. albicans, FKS1 hot spot 1 mutations occur 90% of the time; two loci, S645 and S641, account for 93% of resistance



FKS2 mutations occur at nearly 3 times the rate of FKS1 mutations; S663 (FKS2) and equivalent residue S629 (FKS1) are most prominent



Caspofungin AFST for *Candida* spp. may vary by laboratory and is unreliable

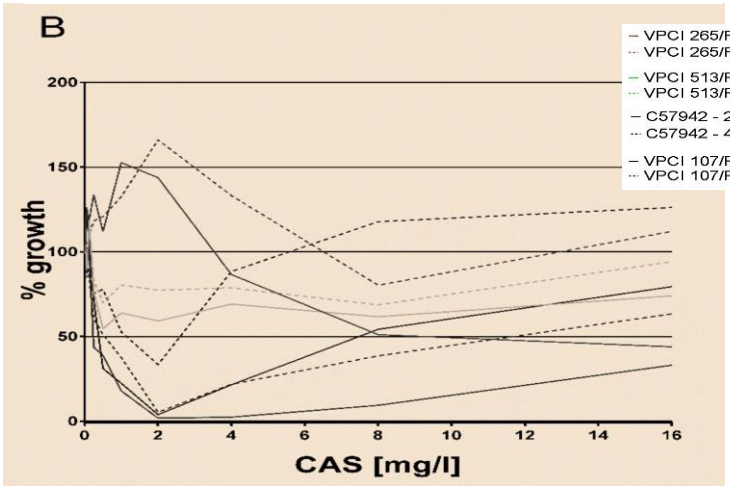
Agent	MIC (µg/ml)	No. of isolates at MIC by laboratory[b]													
		4*	20*	3	2*	18*	19	1	7	11	12	9	14	6	10
Caspofungin	0.0079	10	3			7	92								
	0.016	136	28	181	1	11	1,181				141				
	0.031	15	2	31	755	670	2,037	2	6	15	343				38
	0.063	6	3	55	659	544	898	39	6	59	218	1	20		15
	0.125	8	1	69	357	49	68	24	128	72	345	332	39	58	9
	0.25	6	3	14	212	2	6	10	12	8	117			97	106
	0.5	4		1	176	5	1	7	16	11	71	108			552
	1			3	30	5		1	10	1	10	3	1	13	10
	2				16	2					1			1	3
	4				4							3			
	8				10										
Anidulafungin	0.0079		2	2		260	338	20			121	11			
	0.016	24	306	44	519	1,278	50				252	128			
	0.031	7	45	81	426	1,542	160			609	321	248	59		683
	0.063	5	3	6	164	896	80				209	52			12
	0.125	2		4	18	216	20		2		151	4			8
	0.25	1		3	3	12	5		1		108	1			4
	0.5	1	2		2		1				66				23
	1		2		4	1					13	1	1		1
	2		3		1							2			2

15X

Caspofungin modal MIC variability among independent laboratories using CLSI broth microdilution method for *Candida* spp. (A. Espinel-Ingroff et al. AAC 2013)

Micafungin and anidulafungin behave more reliably in AFST
(Arendrup et al. Mycoses 2014; Pfaller et al. JCM 2014)

Caspofungin Paradoxical Growth in *C. auris*



CAS testing

- 'Eagle effect' (paradoxical growth effect)
- Difficult reading
- AFST with CSF should be viewed cautiously or avoided

Kordalewska et al. 2018 AAC

ECHINOCANDINS - *FKS1* gene analysis

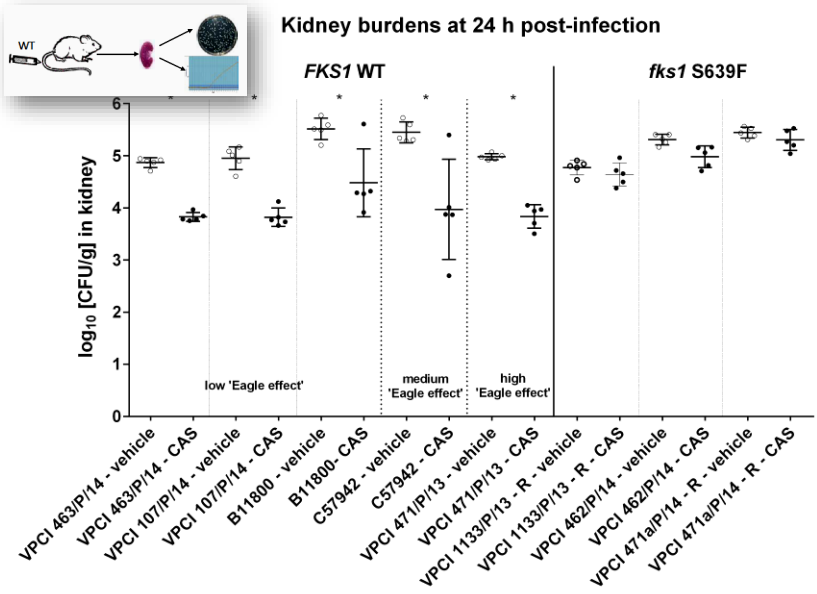
- 102 echinocandin sensitive isolates: wild-type genotype
- Echinocandin-resistant isolates: S639F

Species	Fks1 HS1
C. auris	F$_{635}$L T L **S** L R D P
C. albicans	**F**$_{641}$L T L **S** L R D P
C. glabrata	**F**$_{625}$L I L **S** I R D P
C. parapsilosis	F$_{652}$L T L S L R D **A**
C. tropicalis	**F**$_{650}$L T L **S** L R D P

Most common site for high-level echinocandin resistance

Milena **KORDALEWSKA**

Echinocandin resistance associated with *FKS* genotype



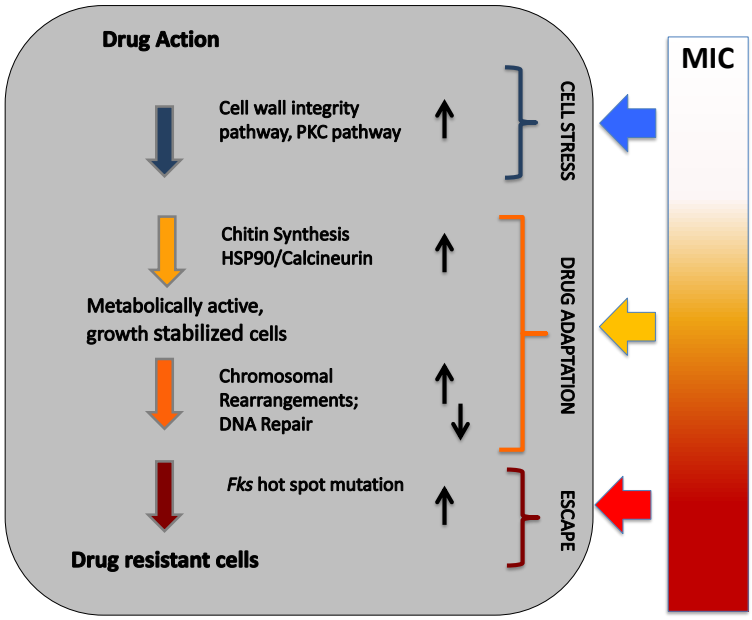
Kordalewska et al. 2018 AAC

Azoles and echinocandins have non-overlapping mechanisms of resistance

Mechanism of resistance	Azoles	Echinocandins
Target site modification		
ERG11	✔	
FKS		✔
Target site overexpression	✔	
Drug pumps	✔	

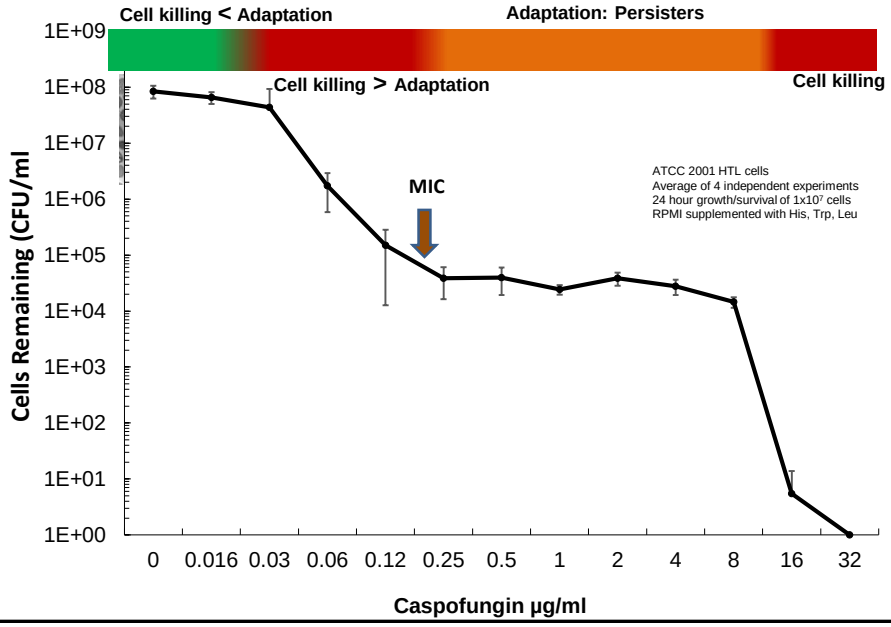
If azoles and echinocandins have distinct non-overlapping mechanisms of resistance, then what is driving multidrug resistance in *C. glabrata*?

Evolution of Echinocandin Resistance

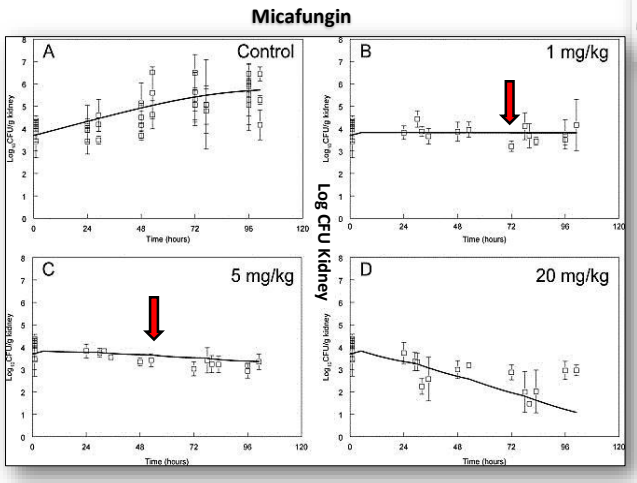


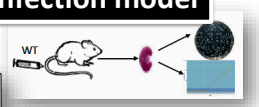
Perlin 2015 Antimicrob Ther Rev

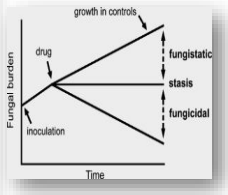
Phases of *in vitro* cell killing and persistence with echinocandins and *Candida glabrata*



Echinocandins are fungicidal yet they act as fungistatic agents in the human therapeutic range in a murine kidney infection model



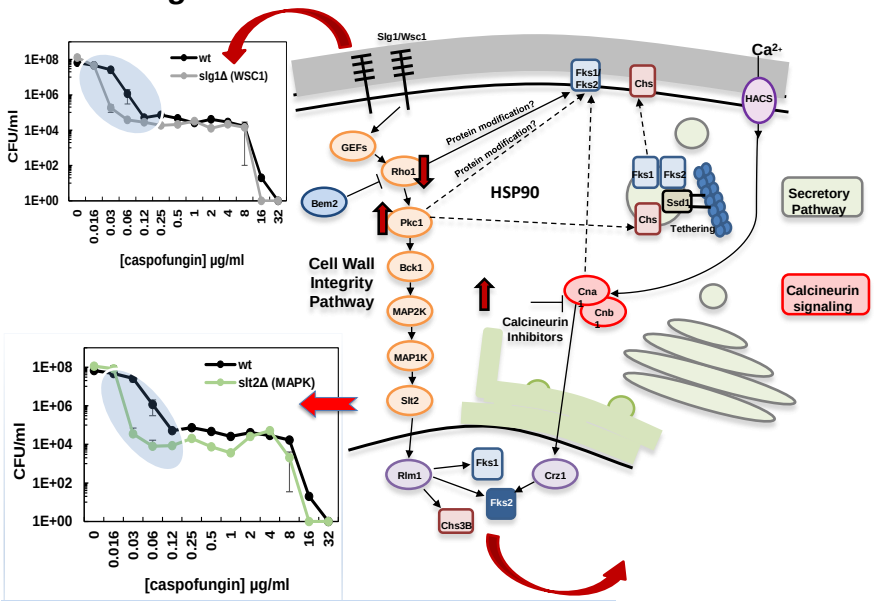




Static behavior reflects cell adaptation due to drug stress

Howard et al. 2011 AAC

Candida glabrata mechanisms of echinocandin tolerance

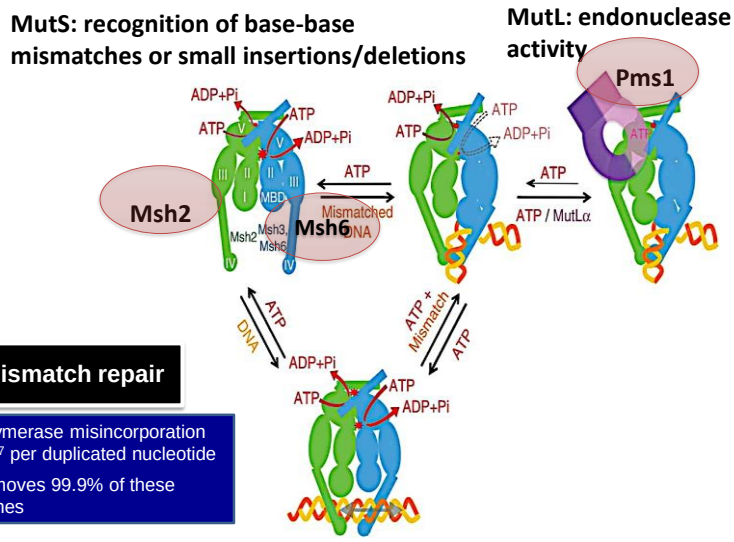


Disruption of tolerance-associated genes or pathways lead to increased caspofungin killing

Healey and Perlin, JoF 2018

How do we reconcile the emergence of two independent resistance mechanisms

An underlying defect that increases spontaneous mutation rates would allow *C. glabrata* to escape drug action by inducing critical independent resistance mechanisms in real time

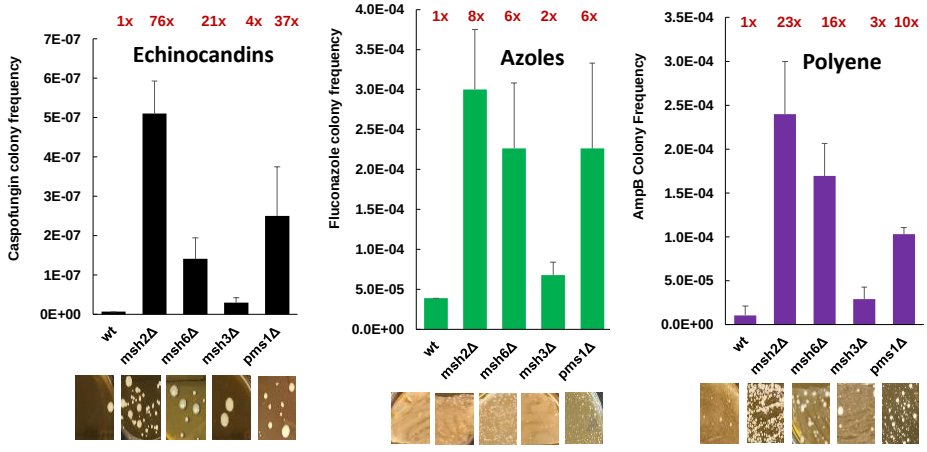


DNA mismatch repair

- DNA polymerase misincorporation rate ~10^{-7} per duplicated nucleotide
- MMR removes 99.9% of these mismatches

Gupta et al., 2012 *Nat Struct Mol Biol*

C. glabrata msh2 Δ, msh6Δ, msh3Δ and *pms1Δ* lead to increased frequencies of antifungal-resistant colonies



Fks1/Fks2 hotspot mutations

Pdr1 GOF mutations

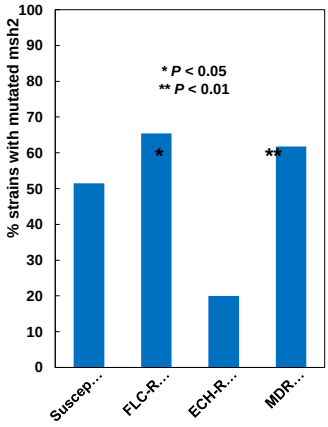
Erg6 mutations

Mutations in all four components enhance selection for MDR phenotypes

Healey et al. 2016 Nat Comm/Unpubl

Msh2 nonsynonymous mutations identified in 55% of 440 geographically diverse, clinical isolates

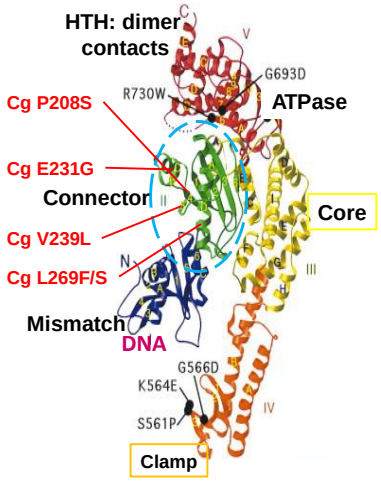
Center	n strains carrying *msh2* mutation/total n (%)			
	Susceptible	FLC-R	ECH-R	MDR
MD Anderson Cancer Center	4/14 (29)	9/9 (100)	0/1 (0)	0/1 (0)
Univ. Hospital Lausanne (Switzerland)	13/25 (52)	11/15 (73)	-	-
Hamad Med. Corp. (Qatar)	21/38 (55)	20/34 (59)	-	2/2 (100)
Duke Hospital	19/36 (53)	6/13 (46)	2/8 (25)	4/8 (50)
Centers for Disease Control	52/99 (53)	0/2 (0)	-	9/17 (53)
Wayne State Univ.	7/9 (78)	7/9 (78)	-	-
Miscellaneous	1/4 (25)	1/1 (100)	1/6 (17)	6/6 (100)
Univ. Delhi (India)	41/82 (51)	1/1 (100)	-	-
TOTAL	158/307 (52)	55/84 (65)	3/15 (20)	21/34 (62)



Healey et al. 2016 Nat Comm

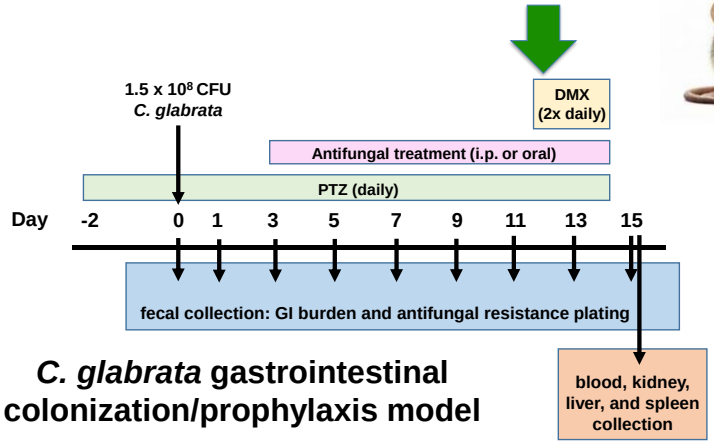
C. glabrata clinical isolates' *msh2* mutations map to the connector domain

Msh2 connector domain essential for interaction with the MutL complex and MMR activity
(Mendillo et al., 2009 PNAS)



Bacterial Msh2 crystal structure:
Meindert et al., 2000 *Nature*; Obmolova et al., 2000 *Nature*

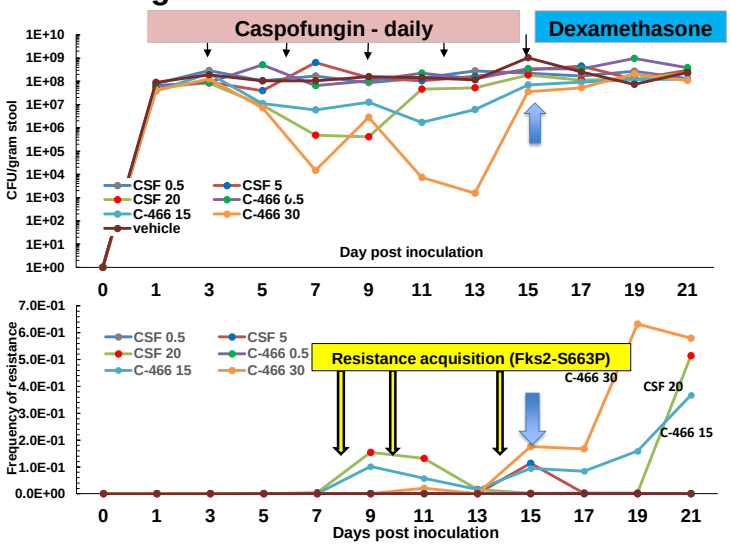
GI tract model for breakthrough resistance



***C. glabrata* gastrointestinal colonization/prophylaxis model**

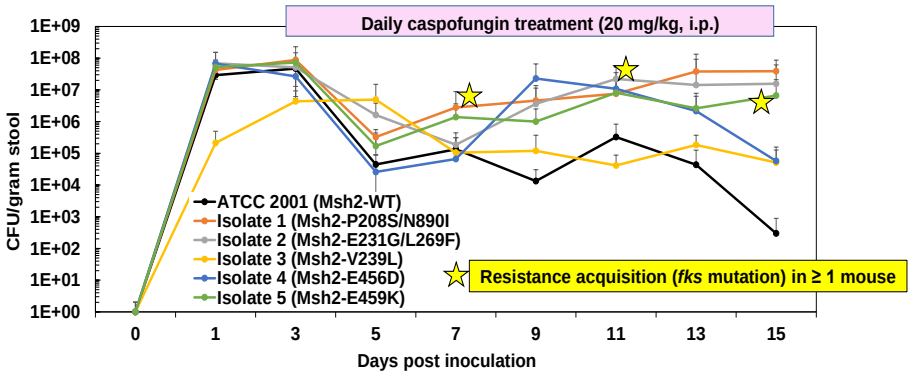
Healey et al. 2017 AAC; Healey et al. 2016 Nat Comm

High GI tract drug exposure leads to resistance among colonized organisms



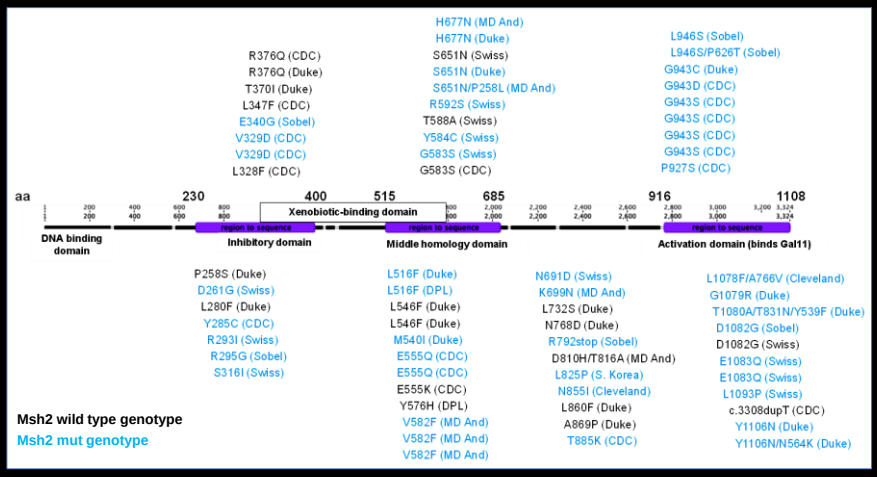
Immunosuppression breaches GIT leading to systemic infection with resistant isolates

Clinical isolates harboring specific Msh2 alleles show enhanced capacity to develop drug resistance



Average GI burdens of mice colonized with the indicated strain and treated with high dose (20 mg/kg) of caspofungin.
Note: 5 mg/kg of CSF is considered the equivalent humanized dose.

pdr1 GOF mutations identified within fluconazole-resistant clinical isolates- *MSH2* prevalence



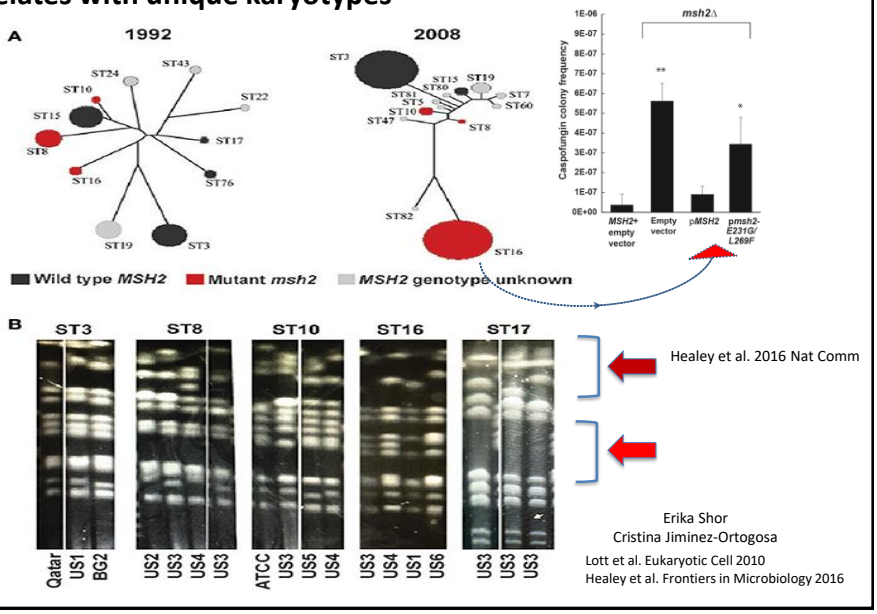
Healey, unpubl

Mismatch repair is not an absolute driver in all patients populations for mono-resistance but appears to be more strongly linked to MDR phenotypes and resistance sequence types

Fluconazole and Echinocandin Resistance of *Candida glabrata* Correlates Better with Antifungal Drug Exposure Rather than with *MSH2* Mutator Genotype in a French Cohort of Patients Harboring Low Rates of Resistance.
Dellière S, Healey K, Gits-Muselli M, Carrara B, Barbaro A, Guigue N, Lecefel C, Touratier S, Desnos-Ollivier M, **Perlin DS**, Bretagne S, Alanio A.
Front Microbiol. 2016 Dec 23;7:2038.

Front Microbiol. 2018 Jul 13;9:1523. doi: 10.3389/fmicb.2018.01523. eCollection 2018.
Multilocus Sequence Typing (MLST) Genotypes of *Candida glabrata* Bloodstream Isolates in Korea: Association With Antifungal Resistance, Mutations in Mismatch Repair Gene (Msh2), and Clinical Outcomes.
Byun SA[1], Won EJ[1], Kim MN[2], Lee WG[3], Lee K[4], Lee HS[5], Uh Y[6], Healey KR[7], Perlin DS[7], Choi MJ[1], Kim SH[1], Shin JH[1].

Different *C. glabrata* sequence types (ST) carry various *msh2* alleles and may have different capacity for evolving drug resistance- ST correlates with unique karyotypes

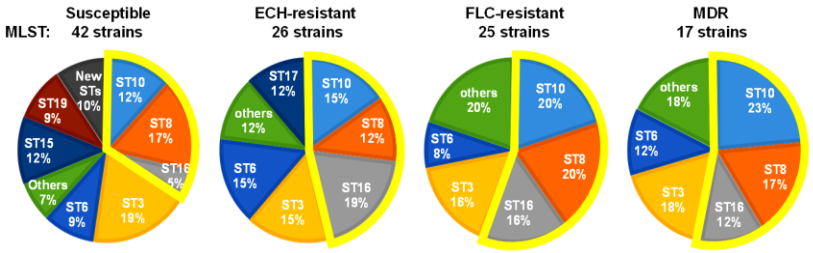


Healey et al. 2016 Nat Comm

Erika Shor
Cristina Jiminez-Ortogosa

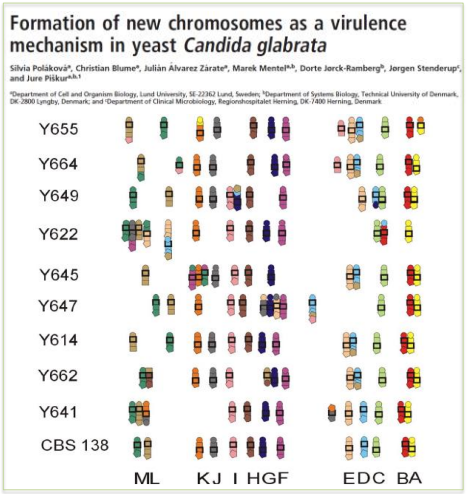
Lott et al. Eukaryotic Cell 2010
Healey et al. Frontiers in Microbiology 2016

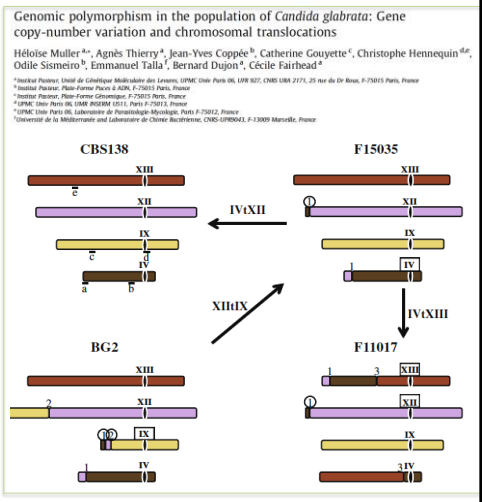
Sequence type distribution of U.S.-based isolates



Sequence type (ST) percentages of susceptible, fluconazole-resistant, echinocandin-resistant, and multidrug (MDR) resistant strains isolated from various U.S. centers. Yellow highlight encompasses STs with partial LOF Msh2 alleles (see Table 1). ST10 and ST16 are nearly exclusive to U.S. isolates

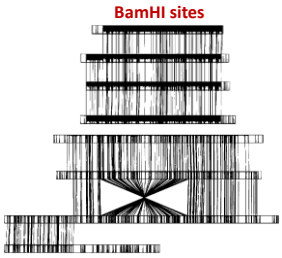
Genomic plasticity in *C. glabrata* aids genetic diversity





To define the genomes of prevalent *C. glabrata* ST's, we performed:

- **PacBio WG sequencing of clinical strains of 7 predominant STs from different hospitals in the US and from Qatar (CDC)**
- **Optical restriction mapping of the same strains (CDC)**

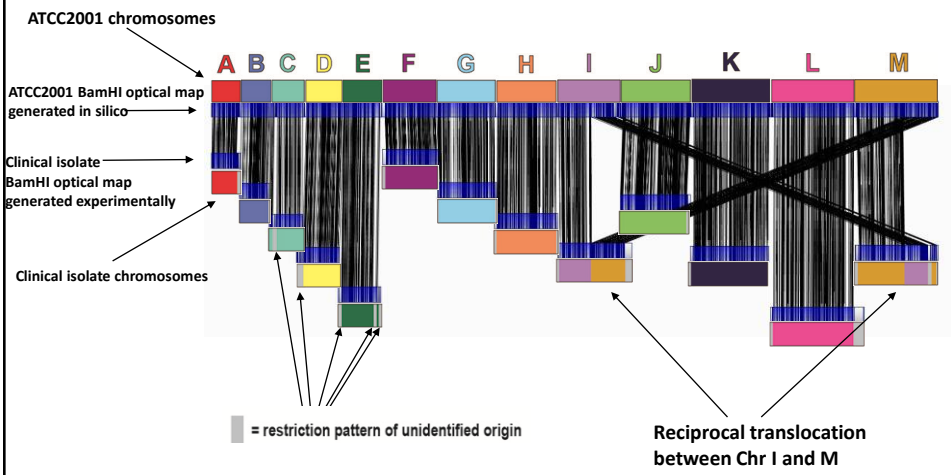


C. *glabrata* reference strain (CBS138) Chr. D
C. *glabrata* isolate 1 Chr. D
C. *glabrata* isolate 2 Chr. D
C. *glabrata* isolate 3 Chr. D
C. *glabrata* isolate 1 Chr. L
C. *glabrata* reference strain Chr. L
C. *glabrata* isolate 3 hybrid chromosome L-I with an inversion in the Chr. L-derived region
C. *glabrata* reference strain Chr. I

- **WGS of 25 strains: 6 predominant STs (Duke collection), ST46 (Qatar), and Indian isolates of new STs**

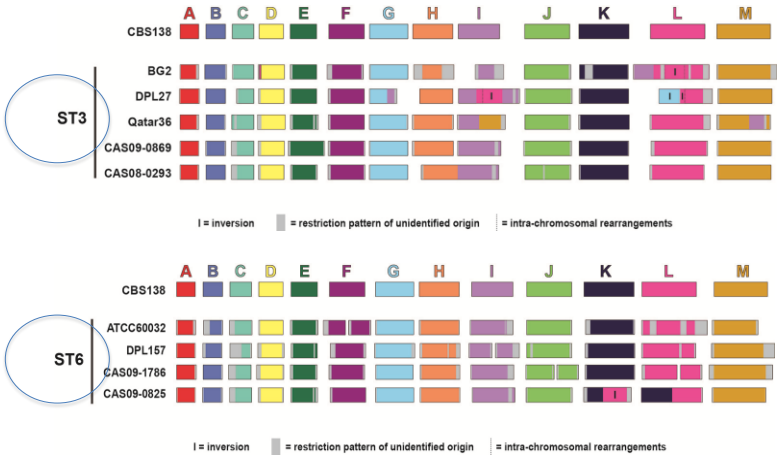
Erika Shor PHRI-Rutgers)
Vladimir Loparev (CDC/OID/NCEZID)
Shawn Lockhart (CDC/OID/NCEZID)

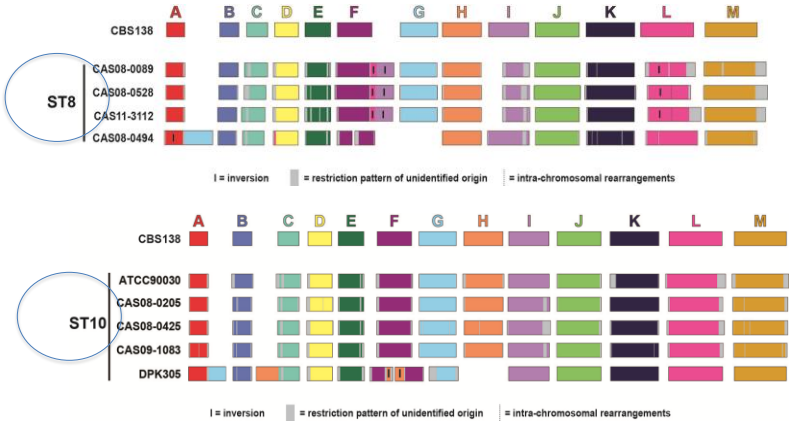
Deep dive chromosomal analyses using linear optical mapping and and WGS of *C. glabrata* ST



Erika Shor PHRI-Rutgers)
Vladimir Loparev (CDC/OID/NCEZID)
Shawn Lockhart (CDC/OID/NCEZID)

Optical maps and long-read PacBio sequencing reveals numerous crossover and recombinational events that define sequence types





Erika Shor PHRI-Rutegrs)
Loparev, Vladimir (CDC/OID/NCEZID)
Shawn Lockhart (CDC/OID/NCEZID)

Preliminary conclusions from optical mapping

- Optical mapping detect large scale genome changes
- Significant rearrangements detected between STs and within STs
- Sub-telomeric regions are highly subject to changes that result in scrambling of restriction patterns and appearance of novel patterns.
- **Maximizing genetic diversity through chromosomal plasticity is a hallmark of *C. glabrata* and other fungal species.**

Summary

- Overall rates of antifungal resistance among *Candida* species are low
- Drug resistance is a growing problem with *C. glabrata*
- DNA mismatch repair (MMR) defects lead to greater frequencies of antifungal-resistant mutants- may be a factor for genetic diversity
- Certain clades of *C. glabrata* carry a greater potential for resistance development
- The ability of *C. glabrata* to maximize genetic diversity allows for rapid adaptable to drugs and environment

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RUTGERS New Jersey Medical School

PHRI

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NIH National Institute of Allergy and Infectious Diseases

CDC

astellas