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

<table>
<thead>
<tr>
<th>Non-microbiological</th>
<th>Microbiological</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Unfavorable PK</td>
<td>✓ Drug resistance</td>
</tr>
<tr>
<td>✓ Poor drug penetration</td>
<td>• Inherent</td>
</tr>
<tr>
<td>✓ Co-morbidities</td>
<td>• Acquired</td>
</tr>
<tr>
<td>✓ Host reservoirs</td>
<td></td>
</tr>
<tr>
<td>• GI tract; intra-abdominal abscesses</td>
<td></td>
</tr>
</tbody>
</table>

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

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

Susceptibility remains high among most Candida species

Resistance among Candida species is low

**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**, 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**

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**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 A1, Healey KR2, Yadav P1, Upadhya G1, Sachdeva N1, Sarma S4, Kumar A5, Taral B5, Perlin DS, Chowdhary A2.

**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 V239D/Q386E. 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).


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.

CHINA


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, Sendergaard 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.
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
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](image)

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

<p>| Table 1. Distribution of Different Candida Species Among Patients With Hematologic Malignancies and Solid Tumors |
|---------------------------------------------------------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Candida species</th>
<th>No. of patients (%)</th>
<th>HM</th>
<th>ST</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>38 (14)</td>
<td>161 (45)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>86 (31)</td>
<td>64 (18)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>68 (24)</td>
<td>6 (2)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>39 (14)</td>
<td>71 (20)</td>
<td>.045</td>
<td></td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>27 (10)</td>
<td>30 (9)</td>
<td>.620</td>
<td></td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td>4 (1)</td>
<td>2 (1)</td>
<td>.410</td>
<td></td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td>3 (1)</td>
<td>3 (1)</td>
<td>.990</td>
<td></td>
</tr>
<tr>
<td>Mixed Candida species</td>
<td>16 (6)</td>
<td>17 (5)</td>
<td>.620</td>
<td></td>
</tr>
</tbody>
</table>

Hachem et al. 2008 Cancer
Concomitant rise in azole and echinocandin resistance among *Candida glabrata* isolates with emergence of multidrug (MDR) resistance

**MDR increasingly more common**

146 Total Isolates
- 20.5% FLUr
- 10.3% CSFr
- 6.8% MDR (CSF, FLU, AMB)

*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


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

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

### Azole resistance mechanisms

**I. Alteration of Drug Target**

<table>
<thead>
<tr>
<th><strong>Erg11</strong></th>
<th><strong>Erg5</strong></th>
<th><strong>Erg11</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida</em> spp.</td>
<td><em>Candida</em> spp.</td>
<td><em>Cr. neoformans</em></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th><strong>ABC</strong></th>
<th><strong>MFS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdr1, <em>C. albicans</em></td>
<td>Mdr1, <em>C. albicans</em></td>
</tr>
<tr>
<td>Cdr1, <em>C. glabrata</em></td>
<td>Flu1, <em>C. albicans</em></td>
</tr>
<tr>
<td>Pdh1, <em>C. glabrata</em></td>
<td>Mcm1, <em>C. albicans</em></td>
</tr>
<tr>
<td>Mdr1,2,4, <em>A. fumigatus</em></td>
<td>Mdr3, <em>A. fumigatus</em></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th><strong>Erg11</strong></th>
<th><strong>Cyp51A</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida</em> spp.</td>
<td><em>Aspergillus</em> spp.</td>
</tr>
</tbody>
</table>

**IV. Chromosomal abnormalities**

- Aneuploidy, disomy, isochromosomes
  - *C. albicans*, *Cr. neoformans*
  - *Aspergillus* spp.
  - e.g. *ERG11, TAC1*

**V. Heteroresistance**

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

**V. Transcription factors**

- TAC1, MRR1, PDR1
- UPC2
Azole resistance in *Candida glabrata*: a perfect storm for multidrug resistance

**GOF Mutations in TF Pdr1**

- **Azole resistance**
  - **Upregulation of CDR1**
  - **Increased burden**
    - Decreased adherence and uptake by macrophages

*Vermitsky et al. 2006 MM*

*Vale-Silva et al. 2013 Infect Immun*

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**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)

<table>
<thead>
<tr>
<th></th>
<th>Erg11</th>
<th>Prevalence</th>
<th>Elevated MICs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>2.5% (n=1)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Y132F</td>
<td>55% (n=22)</td>
<td>FL, VRC, IVC</td>
</tr>
<tr>
<td></td>
<td>K143R</td>
<td>42.5% (n=17)</td>
<td>FLC</td>
</tr>
</tbody>
</table>

### Colombia (n=56)

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>62.5% (n=35)</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y132F</td>
<td>3.5% (n=2)</td>
<td>FL, VRC, IVC</td>
</tr>
<tr>
<td></td>
<td>K143R</td>
<td>1.8% (n=1)</td>
<td>FLC</td>
</tr>
<tr>
<td></td>
<td>I466M</td>
<td>30.4% (n=17)</td>
<td>FLC</td>
</tr>
<tr>
<td></td>
<td>Y501H</td>
<td>1.8% (n=1)</td>
<td>FL, VRC, IVC</td>
</tr>
</tbody>
</table>

Kordalewska 2018

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**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

<table>
<thead>
<tr>
<th></th>
<th>FLC 48h</th>
<th>FLC 72h</th>
<th>VRC 48h</th>
<th>VRC 72h</th>
</tr>
</thead>
<tbody>
<tr>
<td>BY4741 + empty</td>
<td>8</td>
<td>16</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>BY4741 + pCauErg11-WT</td>
<td>16</td>
<td>16</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>BY4741 + pCauErg11-Y132F</td>
<td>128</td>
<td>&gt;128</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>BY4741 + pCauErg11-K143R</td>
<td>64</td>
<td>&gt;128</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>BY4741 + pCauErg11-K177R/N3555/E3433D</td>
<td>8</td>
<td>16</td>
<td>0.12</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*Sagatova et al., 2015 AAC*

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

*HEALEY et al. AAC 2018*
• 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

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

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.

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

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

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

<table>
<thead>
<tr>
<th>Variable:</th>
<th>% sensitivity</th>
<th>% specificity</th>
<th>Likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSI breakpoints (intermediate isolates considered to be resistant)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspofungin BP &gt; 0.12 μg/ml</td>
<td>100 (10/10)</td>
<td>0 (0/29)</td>
<td>NA</td>
</tr>
<tr>
<td>Anidulafungin BP &gt; 0.12 μg/ml</td>
<td>30 (3/10)</td>
<td>97 (28/29)</td>
<td>8.7</td>
</tr>
<tr>
<td>Micafungin BP &gt; 0.06 μg/ml</td>
<td>30 (3/10)</td>
<td>90 (26/29)</td>
<td>2.9</td>
</tr>
<tr>
<td>CLSI breakpoints (intermediate isolates considered to be sensitive)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspofungin BP &gt; 0.25 μg/ml</td>
<td>100 (10/10)</td>
<td>0 (0/29)</td>
<td>NA</td>
</tr>
<tr>
<td>Anidulafungin BP &gt; 0.25 μg/ml</td>
<td>30 (3/10)</td>
<td>97 (28/29)</td>
<td>8.7</td>
</tr>
<tr>
<td>Micafungin BP &gt; 0.12 μg/ml</td>
<td>20 (2/10)</td>
<td>97 (28/29)</td>
<td>5.8</td>
</tr>
<tr>
<td>Epidemiologic cutoff values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspofungin BP &gt; 0.12 μg/ml</td>
<td>100 (10/10)</td>
<td>0 (0/29)</td>
<td>NA</td>
</tr>
<tr>
<td>Anidulafungin BP &gt; 0.25 μg/ml</td>
<td>30 (3/10)</td>
<td>97 (28/29)</td>
<td>8.7</td>
</tr>
<tr>
<td>Micafungin BP &gt; 0.03 μg/ml</td>
<td>40 (4/10)</td>
<td>90 (26/29)</td>
<td>3.9</td>
</tr>
<tr>
<td>Cutoffs determined by ROC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspofungin MIC &gt; 0.5 μg/ml</td>
<td>60 (6/10)</td>
<td>86 (25/29)</td>
<td>4.3</td>
</tr>
<tr>
<td>Anidulafungin BP &gt; 0.03 μg/ml</td>
<td>50 (5/10)</td>
<td>97 (28/29)</td>
<td>14.5</td>
</tr>
<tr>
<td>Micafungin BP &gt; 0.03 μg/ml</td>
<td>40 (4/10)</td>
<td>90 (26/29)</td>
<td>3.9</td>
</tr>
<tr>
<td>FKS genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of mutation</td>
<td>60 (6/10)</td>
<td>97 (28/29)</td>
<td>17.4</td>
</tr>
</tbody>
</table>

Shields et al. AAC 2012
### Spectrum of Fks amino acid changes conferring clinical resistance - 17 year view

<table>
<thead>
<tr>
<th>Organism</th>
<th>Hot Spot1</th>
<th>Fks1</th>
<th>Hot Spot2</th>
<th>Fks2</th>
<th>Hot Spot1</th>
<th>Fks3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>LTLSLRDP</td>
<td>DWIRYTL NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>C. krusei</td>
<td>LTLSLRDP</td>
<td>DWIRYTL NO</td>
<td>LO</td>
<td>NO</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>C. glabrata</td>
<td>LTLSLRDP</td>
<td>DWIRYTL NO</td>
<td>LO</td>
<td>NO</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>LTLALSRDP</td>
<td>DWIRYTL NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>C. lypolytica</td>
<td>LTLALSRDP</td>
<td>DWIRYTL NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>LTLIRDP</td>
<td>DWIRYTL NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td></td>
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<td>C. dubliniensis</td>
<td>LTLIRDP</td>
<td>DWIRYTL NO</td>
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<td>DWIRYTL NO</td>
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</table>

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

---

**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**

Caspofungin

Anidulafungin

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.

**Candida albicans** (n=47 strains with mutations in *FKS1* gene)

- **FKS1 HS1 mutations**
  - 90% HS1
  - 28% F641
  - 65% S645

- **FKS1 HS2 mutations**
  - R1361H
  - R1361R/H
  - R1360R/K
  - 20%

FKS1 mutations occur 90% of the time; two loci, S645 and S641, account for 93% of resistance.

FKS1 HS2 mutations occur at nearly 3 times the rate of FKS1 mutations; S663 (FKS2) and equivalent residue S629 (FKS1) are most prominent.

**Candida glabrata** (n=93 strains with mutations in *FKS* genes)

- **FKS1**
  - 26%

- **FKS2**
  - 74%

**Mutations found in FKS1**
- D632Y
- F625S
- S629P
- D632G
- D632E
- I634V
- R631G
- L660F
- D632Y
- F659V
- F659S
- delF659
- others HS1
- HS2

**Mutations found in FKS2**
- S663P
- F659V
- F659S
- delF659
- others HS1
- HS2

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

<table>
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<tr>
<th>Agent</th>
<th>MIC (μg/ml)</th>
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<th>20&quot;</th>
<th>3&quot;</th>
<th>2&quot;</th>
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<th>11</th>
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</table>

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*

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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

<table>
<thead>
<tr>
<th>Species</th>
<th>Fks1 HS1</th>
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<tbody>
<tr>
<td>C. auris</td>
<td>F_{635}LTLSSLRDP</td>
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<tr>
<td>C. albicans</td>
<td>F_{641}LTLSSLRDP</td>
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<td>C. glabrata</td>
<td>F_{625}LILSIRDP</td>
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<td>C. parapsilosis</td>
<td>F_{652}LTLSSLRDA</td>
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<tr>
<td>C. tropicalis</td>
<td>F_{650}LTLSSLRDP</td>
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</table>

Most common site for high-level echinocandin resistance

Milena KORDALEWSKA

Echinocandin resistance associated with FKS genotype

Kidney burdens at 24 h post-infection

Kordalewska et al. 2018 AAC
Azoles and echinocandins have non-overlapping mechanisms of resistance

<table>
<thead>
<tr>
<th>Mechanism of resistance</th>
<th>Azoles</th>
<th>Echinocandins</th>
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<tr>
<td>Target site modification</td>
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<td>✔</td>
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<tr>
<td>Target site overexpression</td>
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<tr>
<td>Drug pumps</td>
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</table>

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*

ATCC 2001 HTL cells
Average of 4 independent experiments
24 hour growth/survival of 1x10^7 cells
RPMI supplemented with His, Trp, Leu

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

Howard et al. 2011 AAC

Static behavior reflects cell adaptation due to drug stress
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 $\sim 10^{-7}$ per duplicated nucleotide
- MMR removes 99.9% of these mismatches

**C. glabrata msh2Δ, msh6Δ, msh3Δ and pms1Δ lead to increased frequencies of antifungal-resistant colonies**

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

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

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)
**GI tract model for breakthrough resistance**

1.5 x 10^8 CFU C. glabrata

Antifungal treatment (i.p. or oral)

PTZ (daily)

Day -2 0 1 3 5 7 9 11 13 15

fecal collection: GI burden and antifungal resistance plating

**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**

Caspofungin - daily

Dexamethasone

Resistance acquisition (Fks2-S663P)

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.**

**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.**

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


Collaborators

- **S.R. Lockhart**: U.S. Centers for Disease Control and Prevention
- **D.P. Kontoyiannis**: MD Anderson CC
- **B. Alexander**: Duke University
- **D. Sanglard**: University Hospital of Lausanne
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- **Anuradha Chowdhary**: New Delhi
- **Alexandre Alaino**: Pasteur Institute

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