

Genomic description of acquired fluconazole- and echinocandin-resistance in patients with serial *Candida glabrata* isolates

E. Misas,¹ E. Seagle,¹ E. N. Jenkins,^{1,2} M. Rajeev,¹ S. Hurst,¹ N. S. Nunnally,¹ M. L. Bentz,¹ M. M. Lyman,¹ E. Berkow,¹ L. H. Harrison,³ W. Schaffner,⁴ T. M. Markus,⁴ R. Pierce,⁵ M. M. Farley,⁶ N. A. Chow,¹ S. R. Lockhart,¹ A. P. Litvintseva¹

AUTHOR AFFILIATIONS See affiliation list on p. 11.

ABSTRACT *Candida glabrata* is one of the most common causes of systemic candidiasis, often resistant to antifungal medications. To describe the genomic context of emerging resistance, we conducted a retrospective analysis of 82 serially collected isolates from 33 patients from population-based candidemia surveillance in the United States. We used whole-genome sequencing to determine the genetic relationships between isolates obtained from the same patient. Phylogenetic analysis demonstrated that isolates from 29 patients were clustered by patient. The median SNPs between isolates from the same patient was 30 (range: 7–96 SNPs), while unrelated strains infected four patients. Twenty-one isolates were resistant to echinocandins, and 24 were resistant to fluconazole. All echinocandin-resistant isolates carried a mutation either in the *FKS1* or *FKS2* HS1 region. Of the 24 fluconazole-resistant isolates, 17 (71%) had non-synonymous polymorphisms in the *PDR1* gene, which were absent in susceptible isolates. In 11 patients, a genetically related resistant isolate was collected after recovering susceptible isolates, indicating *in vivo* acquisition of resistance. These findings allowed us to estimate the intra-host diversity of *C. glabrata* and propose an upper boundary of 96 SNPs for defining genetically related isolates, which can be used to assess donor-to-host transmission, nosocomial transmission, or acquired resistance.

IMPORTANCE In our study, mutations associated to azole resistance and echinocandin resistance were detected in *Candida glabrata* isolates using a whole-genome sequence. *C. glabrata* is the second most common cause of candidemia in the United States, which rapidly acquires resistance to antifungals, *in vitro* and *in vivo*.

KEYWORDS *Candida glabrata*, antifungal, resistance, genomic, WGS, epidemiology

Candida glabrata is a yeast normally found in commensal microbial communities in the human gastrointestinal tract (1, 2), and it is also an opportunistic pathogen (3) that has become the second most common cause of invasive candidiasis in the United States, followed by *C. albicans* (4). The rise of *C. glabrata* and other non-*Candida albicans* *Candida* (NCAC) species is concerning due to their increased resistance to antifungal medications (5–7).

Currently, only three major classes of medications (polyenes, azoles, and echinocandins) are approved for treating invasive fungal infections. Amphotericin B is a polyene antifungal agent with a broad range of activity against yeasts and mold. Toxicity remains a drawback, despite the development of novel, less-toxic, lipid-based polyene formulations. Azoles are active against *Candida* spp., with fluconazole being the most widely prescribed antifungal in the class, but fluconazole often has reduced activity against *C. glabrata* (8, 9). Treatments with echinocandins have shown improved survival and greater clinical success (10), and their fungicidal activity makes them the first choice against *Candida* species (11).

Editor Kimberly E. Hanson, University of Utah, Salt Lake City, Utah, USA

Address correspondence to A. P. Litvintseva, frq8@cdc.gov.

The authors declare no conflict of interest.

See the funding table on p. 11.

The findings and the conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. The use of trade names is for identification only and does not imply endorsement.

Received 6 September 2023

Accepted 29 November 2023

Published 24 January 2024

This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply.

Approximately 10% of *C. glabrata* isolates are resistant to azoles (6). The predominant mechanism of azole resistance in this pathogen is overexpression of genes such as *CgCDR1*, *CgCDR2*, and *CgSNQ2* that encode ATP-binding cassette (ABC) efflux pumps (7, 12). In addition, azole resistance is associated with mutations in the *PDR1* gene, which is a transcription factor for ABC-transporter expression (13, 14).

Resistance to echinocandins is due to substitutions in the *FKS1* and *FKS2* genes that encode subunits of the β -(1,3)-D-glucan synthase enzyme complex. This enzyme catalyzes the production of glucan, the major component in *Candida* cell walls (15). In these proteins, two hotspots for mutation, HS1 and HS2, located in the regions of the enzymes that form a binding pocket for echinocandins, are most frequently associated with resistance in *Candida* species. In *C. glabrata*, mutations in the HS1 hotspot of *FKS2* are the most common mutations (16) and occur at nearly three times the rate of *FKS1* mutations; S663 (*FKS2*) and equivalent residue S629 (*FKS1*) are the most predominant HS1 hotspot mutations (17).

The emergence of echinocandin resistance in *C. glabrata* in response to treatment has been well-documented (18–20). Numerous *in vitro* (21) and *in vivo* (2, 18, 19, 22) studies have confirmed the ability of *C. glabrata* to develop resistance in the presence of echinocandins. Acquired resistance to fluconazole is well-documented (23), and multidrug resistance (MDR), understood here as resistance to at least two classes of antifungal drugs, has been recorded in numerous clinical cases (22, 24, 25). In addition, acquired MDR in isolates of *C. glabrata* has been reported in studies *in vitro* (26).

Most previous studies showing the acquisition of resistance in *C. glabrata* have relied on multilocus sequence typing (MLST), which incorporates only a small fraction of the genome in the analysis to identify isolates with the same genotype (27). A few whole-genome sequencing (WGS) studies have been performed on serial isolates of *C. glabrata* (28) to investigate the acquisition of echinocandin (29) and azole (30) resistance during therapy. However, these previous studies included few patients (28–30) and did not evaluate the genetic relationships among isolates in a systematic way by using a larger collection of serially collected isolates with different genetic backgrounds. Here, we conducted a retrospective analysis of serially collected clinical isolates of *C. glabrata* among patients with >1 case of candidemia as part of the Emerging Infections Program (EIP) population-based candidemia surveillance by the Centers for Disease Control and Prevention (CDC) in the United States. We used WGS to describe the genetic relationships between resistant and susceptible isolates from the same patient, proposed an SNP threshold for defining the clonal relationship between isolates, and demonstrated the acquisition of resistance in response to treatment. These results provide a genomic framework for future outbreak investigations and for studies investigating the nosocomial transmission of *C. glabrata*.

MATERIALS AND METHODS

Isolate collection

Since 2008, the CDC has conducted active, population-based surveillance for candidemia through the Emerging Infections Program in select counties within the United States (<https://www.cdc.gov/ncezid/dpei/eip/index.html>). For surveillance purposes, a case was defined as a positive blood culture for any *Candida* species collected from a patient who resided within the surveillance areas. If collected at autopsy, cultures must have been collected ≤ 12 hours from death. A patient with a positive *Candida* blood culture collected >30 days after the initial positive culture was considered to have a new case. Cases were identified through the inpatient and outpatient laboratories serving the surveillance population. For each case, a trained surveillance officer reviewed medical records to gather demographic information and clinical characteristic data. *Candida* isolates collected from the incident culture for the cases were sent to the CDC for species confirmation and antifungal drug susceptibility testing. For *C. glabrata*, subsequent isolates within the 30-day case period were also collected.

Human subjects

This activity was reviewed by the CDC and was conducted consistent with the applicable federal law and CDC policy (see, e.g., 45 CFR part 46, 21 CFR part 56; 42 USC §241(d); 5 USC §552 a; 44 USC §3501 et seq).

Antifungal susceptibility testing

Antifungal susceptibility testing (AFST) was performed by broth microdilution as described in Clinical and Laboratory Standards (CLSI) M27 ed3 (31). Microdilution plates were custom-made by ThermoFisher and were stored frozen until use. Breakpoints for resistance for *Candida glabrata* were defined according to CLSI as follows: the breakpoint for anidulafungin was ≥ 0.5 $\mu\text{g}/\text{mL}$, that for caspofungin was ≥ 0.5 $\mu\text{g}/\text{mL}$, and that for micafungin was ≥ 0.25 $\mu\text{g}/\text{mL}$. For fluconazole, the breakpoint was ≥ 64 $\mu\text{g}/\text{mL}$.

WGS prioritization

Patients with the >1 *C. glabrata* case (as defined above) between 2008 and 2015 were identified through a CDC candidemia surveillance database query. Using incident isolates only (i.e., only the first isolate for each case), isolates for patients of which at least one was found to be resistant to fluconazole or an echinocandin with MIC values above the defined breakpoints were submitted for whole-genome sequencing to determine the relatedness of strains across cases within each patient. Fourteen patients (42%) with all incident-susceptible isolates were submitted for WGS. Following WGS, patients with unrelated *C. glabrata* strains were excluded (criteria for relatedness among isolates are defined in the results). For patients with >2 cases, if at least two isolates were related, the patient was included based on only the related isolates (Fig. 1).

We also defined a subgroup of patients with susceptible isolates followed by at least one resistant isolate, referred to as “patient with susceptible-to-resistant pattern.” This sub-dataset of 11 patients with related isolates serves to assess if resistance was acquired after treatment with antifungal drugs.

WGS library preparation

DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Gaithersburg, MD, USA) according to the manufacturer's recommendations. Genomic libraries were constructed using the NEBNext Ultra DNA Library Prep kit (New England Biolabs, Ipswich, MA, USA) for Illumina sequencing. They were sequenced on either the Illumina HiSeq 2500 platform using the HiSeq Rapid SBS kit v2 (500 cycles) or the NovaSeq platform using Illumina Nova-Seq using the 6000 Sp reagent kit (500 cycles). Read data were deposited into the Sequence Read Archive (SRA) database from the National Center for Biotechnology Information (NCBI), BioProject [PRJNA1009065](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1009065).

SNP calling and phylogenetic reconstruction

An assembly of *C. glabrata* CBS138 comprising 14 chromosomes, including the mitochondrial chromosome, was used as the reference for read mapping and SNP calling. This reference sequence had a length of 12.3 Mb, GC content of 38.6%, and N50 of 1.1 Mb. For the whole-genome SNP analysis, the MycoSNP workflow (v 0.21) was used (32).

A masked reference was used for phylogenetic reconstruction, and an unmasked reference was used for SNP annotations. The reference genome was masked for repeats using the nucmer command from MUMmer (v 4.0) (33) and Bedtools (v 2.29.2) (34). The reference genome was indexed for read alignment using the BWA index command and for variant calling using Samtools (v 1.10) (35) faidx and Picards GATK (v 2.22.9) [<http://broadinstitute.github.io/picard/>]. Trimming and filtering of low-quality data were performed using FaQCs (v 2.10) (36). The trimmed reads were used for alignment using the BWA (0.7.17) MEM command (37). Furthermore, the aligned BAM files from each sample were pre-processed using Samtools and Picard commands and made ready for

Inclusion Chart

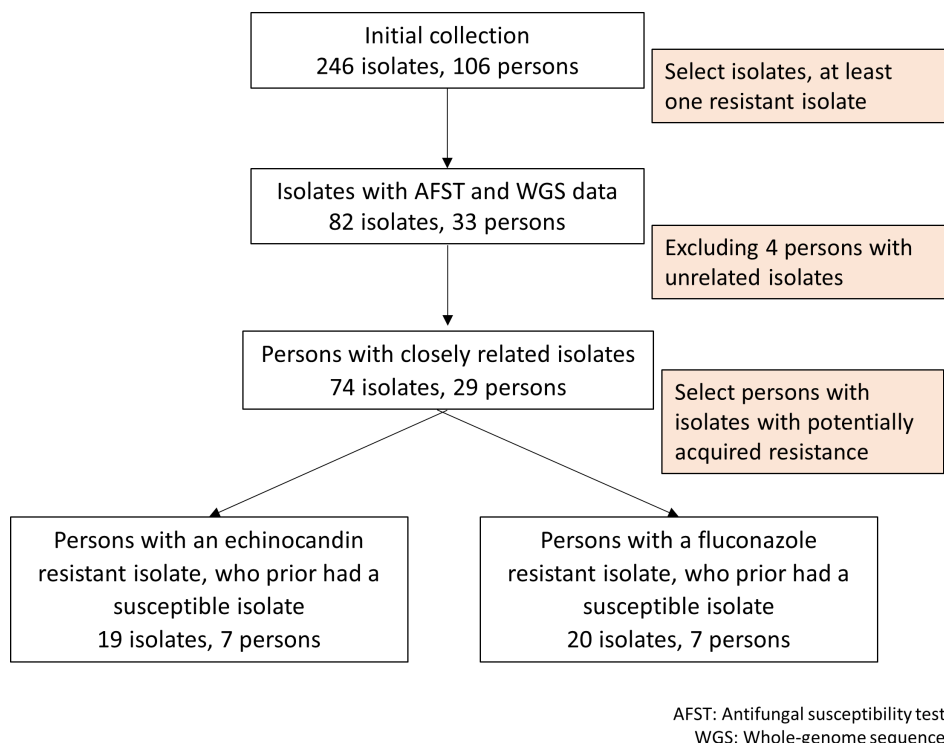


FIG 1 Inclusion chart

variant calling. The Genome Analysis Toolkit (GATK) (v 4.1.4.1) (38) was used for variant calling using the haploid mode. The GATK VariantFiltration tool was used to filter sites based on the filtering expressions “QD <2.0,” “FS >60.0,” and “MQ <40.0.” Using a customized script, genotypes were filtered if the minimum genotype quality was <50, the percentage alternate allele was <0.80, or the depth was <10.

Variable positions in FASTA format were recovered from the VCF files using a Python script “vcfSnpsToFasta.py” (<https://github.com/broadinstitute/broad-fungalgroup/tree/master/scripts/SNPs>). For the phylogenetic analysis, 243,415 sites were concatenated. All positions containing gaps and missing data were eliminated. For the resulting sequences, the pairwise distance, neighbor-joining tree (NJ), and an additional phylogenetic tree with bootstrapping were calculated with the MEGA-X program (39). Parameters for the NJ tree were 1) 1,000 bootstraps, 2) nucleotide substitution transversion model, and 3) unequal base frequency (TVM). For tree visualization, we used the web-based JavaScript application, Microreact (40).

The median SNP differences were calculated for all pairwise comparisons between isolates from the same patient and among different patients using the MEGA-X pairwise distance matrix (39) and in-house R scripts (v 4.0.3) (41).

SNP annotations

The regular VCF output from MycoSNP did not allow us to identify all the mutations in the *FKS2* gene since the gene sequence was masked. To address this issue, the MycoSNP code was modified not to execute the masking step on the reference genome. The VCF file obtained with the modified MycoSNP workflow was used by SnpEff (v 5.0) to predict the effects of associated mutations within genes (42) (Fig. S4). The SNPs were annotated using the database available in SnpEff for *C. glabrata* GCA 000002545. To identify mutations only in the coding regions, we used the parameters “-no-downstream,”

“-no-upstream,” and “-no-intergenic.” Several filters were applied to the annotated VCF to identify the mutations present in some protein-encoded genes associated with resistance to echinocandins (*FKS1* and *FKS2*) and a gene associated with resistance to fluconazole (*PDR1*) at specific positions previously reported in the literature. R in-house scripts were used to compare groups of samples, perform a Wilcoxon statistic test, and construct all R plots.

For the sub-dataset of patients with the susceptible-to-resistant pattern, we used R data frame filters to combine information on susceptibility profile, presence of mutations, collection dates, and clinical data on the treatment with antifungals to identify potential patients with acquired resistance.

RESULTS

Specimen collection and antifungal susceptibility testing

Initially, 246 *C. glabrata* isolates were available from 106 patients. Of those, 82 isolates from 33 patients were prioritized for WGS using the prioritization methods previously described (Fig. 1). The included isolates were obtained from surveillance sites across four states, mainly from Georgia ($n = 33$ isolates) and Maryland ($n = 45$ isolates), plus one more patient from Oregon ($n = 2$ isolates) and another from Tennessee ($n = 2$ isolates). Most (78%) of these 33 patients had two isolates, but two patients had a maximum of five isolates (Table S1).

SNP calling and phylogenetic reconstruction

Phylogenetic analysis revealed that most isolates were clustered by patient (Fig. 2). A total of 243,415 nucleotide positions were used for the neighbor-joining tree method, and the median SNP difference observed between isolates from the same patient was 30 SNPs (range 7–96 SNPs), which was significantly lower than the median SNP difference observed between isolates from different patients (70,448 SNPs; P -value < 0.001 ; Fig. 3).

Genetic clustering by patient was confirmed using a maximum likelihood tree with 1,000 bootstraps (Fig. S1). Isolates from Patient 12 and Patient 18 were grouped in two different clusters (Fig. S2). Only the related isolates were included in the median SNP difference calculation.

Four patients had large SNP differences between isolates (70,294–76,707 SNPs), and one patient (Patient 33) had 555 SNPs (Fig. S2). They were deemed to have multiple genotypes and were excluded from SNP annotation analysis. The remaining 29 patients had smaller SNP differences between isolates (7–96 SNPs) and were considered to have a single genotype (Fig. 2). Of the 29 patients with a single genotype, 11 patients exhibited the susceptible-to-resistant (SR) pattern, including four with a fluconazole-susceptible isolate followed by a fluconazole-resistant isolate (Table 2), four with an echinocandin-susceptible isolate followed by an echinocandin-resistant isolate (Table 1), and three with a susceptible isolate to both followed by an isolate resistant to echinocandins and fluconazole (Fig. 1).

Phylogenetic reconstruction showed no evidence of clustering by geographic site. In addition, time between isolation dates and the genetic distance between serially collected isolates were not correlated (Fig. S3).

SNP annotation related to echinocandin resistance

We analyzed sequences of the *FKS1* and *FKS2* genes extracted from the WGS data in 74 isolates representing 29 patients with a single genotype among all isolates. Twenty-one isolates were resistant to an echinocandin. Twenty of the echinocandin-resistant isolates carried mutations in the hotspot regions of *FKS1* or *FKS2*, while none of the 53 susceptible isolates had mutations in the hotspot regions of these genes. (Table S2).

Of the seven patients in which echinocandin-resistant isolates with the same genotype were isolated after first recovering echinocandin-susceptible isolates

TABLE 1 SR pattern of echinocandin^c

| ID | Patient ID | Date collected | Anid MIC value | Cas MIC value | Mutations FKS1 | Mutations FKS2 | Treatment ^b |
|----------------|------------|----------------|----------------|---------------|----------------|----------------|------------------------|
| CAS10-2614_S20 | 22 | 27-Oct-10 | 0.03 | 0.03 | | | Mica ^a |
| CAS12-3684_S68 | 22 | 21-Nov-11 | 1 | 2 | | Ser663Pro | Mica |
| CAS13-4530_S71 | 22 | 1-Nov-12 | 4 | >16 | | Ser663Pro | Mica; Flu; Ampho |
| CAS13-4778_S42 | 22 | 13-Mar-13 | 0.5 | 0.5 | | Ser663Pro | Mica; Flu; Ampho |
| CAS13-5011_S55 | 22 | 16-Jun-13 | 2 | 8 | | Ser663Pro | Mica; Flu |
| CAS08_0572_S24 | 24 | 13-Oct-08 | 0.125 | 0.125 | | | Unknown |
| CAS08-0725_S45 | 24 | 3-Dec-08 | 1 | 0.5 | | Phe659Try | Unknown |
| CAS09_0869_S25 | 24 | 5-Jan-09 | 1 | 2 | | Phe659Try | Unknown |
| CAS11-2902_S56 | 29 | 31-Jan-11 | 0.03 | 0.06 | | | Mica |
| CAS11-2996_S80 | 29 | 4-Mar-11 | 4 | >16 | | Ser663Pro | Flu; Mica |
| CAS156404_S48 | 31 | 18-Mar-15 | 0.03 | 0.03 | | | Mica |
| CAS156677_S67 | 31 | 6-Aug-15 | 2 | 8 | Ser629Pro | | None |
| CAS08_0124_S32 | 1 | 28-May-08 | 0.03 | 0.03 | | | |
| CAS12-4416_S11 | 1 | 3-Nov-12 | 2 | 2 | Ser629Pro | | Flu; Mica |
| CAS13-5111_S2 | 14 | 9-Oct-13 | 0.03 | 0.03 | | | Mica |
| CAS13-5257_S26 | 14 | 3-Dec-13 | 2 | 8 | | Ser663Pro | Mica |
| CAS14-5368_S62 | 14 | 8-Jan-14 | 2 | 8 | | Ser663Pro | Ampho; Mica |
| CAS14-5293_S38 | 21 | 24-Nov-13 | 0.06 | 0.06 | | | Mica; Flu |
| CAS14-5468_S77 | 21 | 10-Feb-14 | 2 | 4 | Phe625Ser | | Mica; Flu |

^aMica = micafungin, Flu = fluconazole, Anid= anidulafungin, Ampho = amphotericin B.

^bPatient received systemic antifungal medication to treat candidemia on or after positive culture date.

^cPresence of the main reported mutations in HS1 FKS1 and FKS2. Shaded MIC values represent resistant isolates. Patient IDs in bold font represent patients in common with SR pattern for fluconazole.

(susceptible-to-resistant pattern), all had echinocandin-resistant isolates with mutations either in the *FKS1* or *FKS2* HS1 region. Seven echinocandin-resistant isolates from three cases had an S663P substitution in *FKS2*. Two echinocandin-resistant isolates from one case had an F659Y substitution in *FKS2*. Two echinocandin-resistant isolates from two different cases had an S629P mutation in *FKS1*, and one echinocandin-resistant isolate had an F625S mutation in *FKS1* (Table 1; Fig. 4). The median number of SNPs between

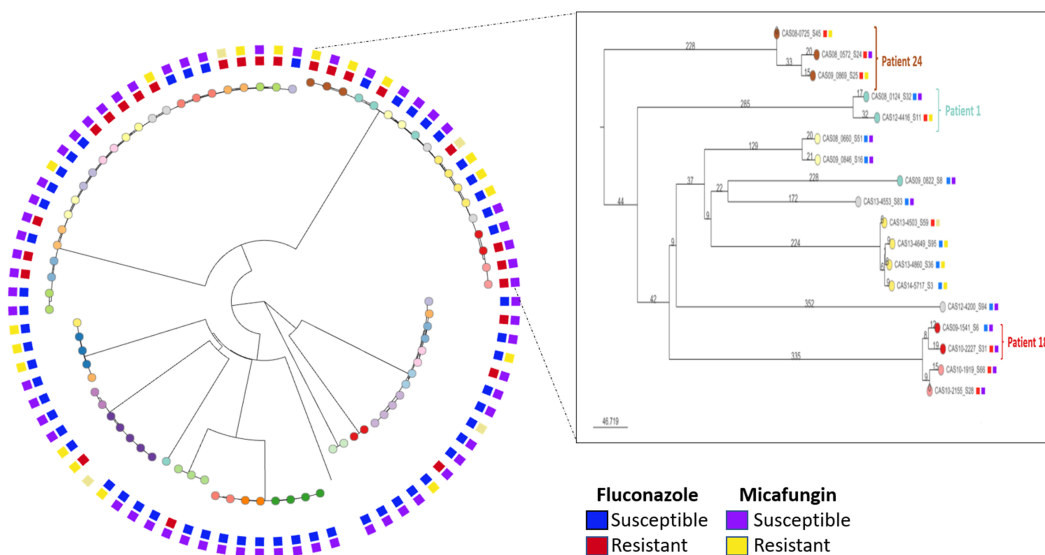


FIG 2 Neighbor-joining phylogeny of *C. glabrata*. The final data set included a total of 243,415 nucleotides for 82 isolates. Each leaf node color represents a patient, and the external squares indicate susceptible/resistant phenotype. Box: zoom in shows examples of patients with isolates that acquired resistance to echinocandins (Patient 24), azoles (Patient 18), or both (Patient 1). Branch length indicates the number of SNPs.

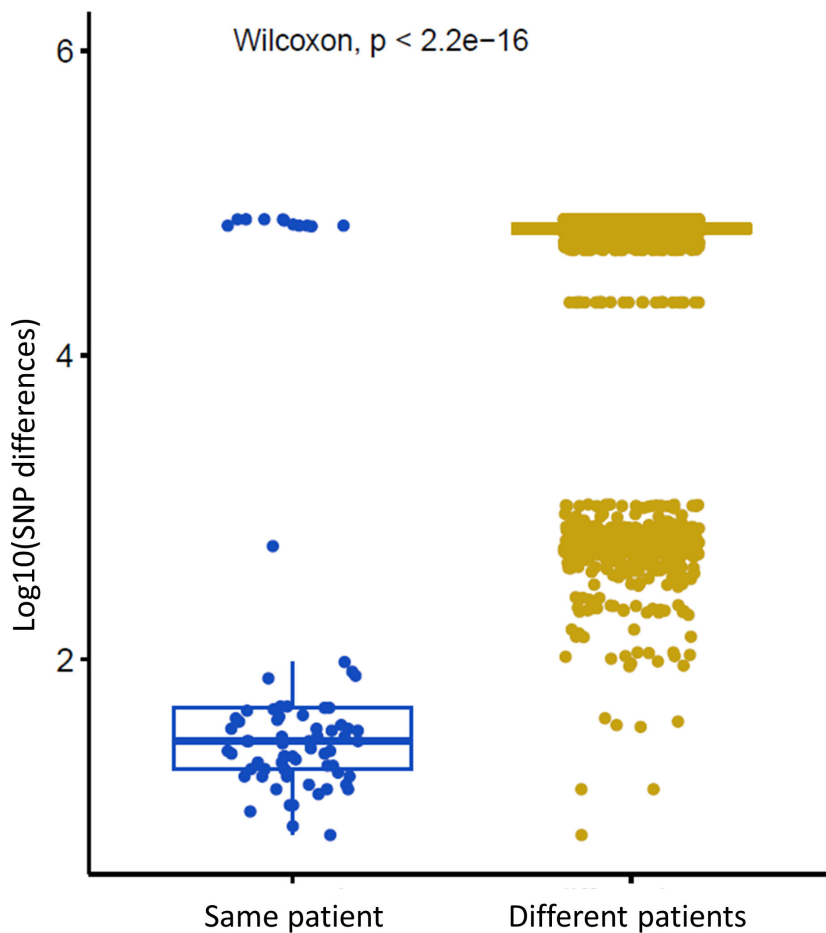


FIG 3 Genetic diversity of *C. glabrata* isolates. Left: SNP differences among several isolates collected from the same patient in 33 patients. Right: SNP differences for each pairwise comparison among all isolates from different patients in 106 patients. Y-axis shows the log 10 of SNP distances. Boxplot R version 4.0.3

susceptible and echinocandin-resistant isolates from the same patient was not significantly different from the median number of SNPs between isolates when the resistance pattern did not change (24 SNPs vs 27 SNPs, P -value = 0.87; Fig. 5).

SNP annotation related to fluconazole resistance

Twenty-five isolates were resistant to fluconazole (MIC ≥ 64 $\mu\text{g/mL}$). We identified nine different non-synonymous polymorphisms in the *PDR1* gene; three of them have been previously reported in the literature to be related to azole resistance (E714D, N109D, and R376Q). These changes were found in 16 (Table S2) of the 25 resistant isolates and were absent in all susceptible isolates.

In seven patients, fluconazole-resistant isolates were isolated after first recovering fluconazole-susceptible isolates (susceptible-resistant pattern). Five of those patients had isolates with different non-synonymous substitutions in the *PDR1* gene. The amino acids differed between the susceptible and resistant paired isolates (Table 2).

Overall, serial isolates from 11 patients showed a susceptible-to-resistant pattern, four for echinocandins, four for fluconazole, and three for both. All 11 patients were treated with antifungals before or after recovery of the first susceptible isolate. Of the four patients whose isolates demonstrated acquired resistance to fluconazole, two received treatment with fluconazole and two were treated with antifungals of an unknown drug class. Of the four patients whose isolates demonstrated acquired resistance to echinocandins, three received treatment with micafungin and one was treated with an

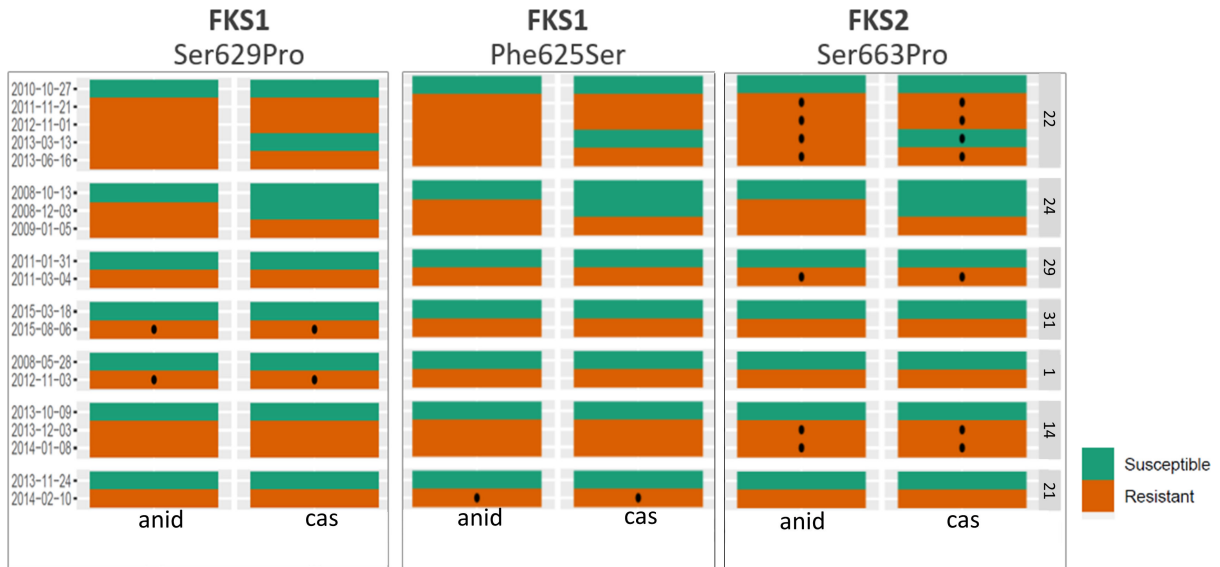


FIG 4 FSK2 and FKS1 common mutations and susceptible/resistant phenotype (graphic representation of Table 1). Red and green boxes represent resistant and susceptible isolates, respectively. Black dots represent the presence of the mutation indicated on the top of each panel. Collection dates are displayed on the right side of the panels and Patient ID on the left. cas: caspofungin, anid: anidulafungin.*echinocandin-resistant isolate from Patient 24 carried the mutation Phe659Try in the *FKS2* gene, not shown in the figure

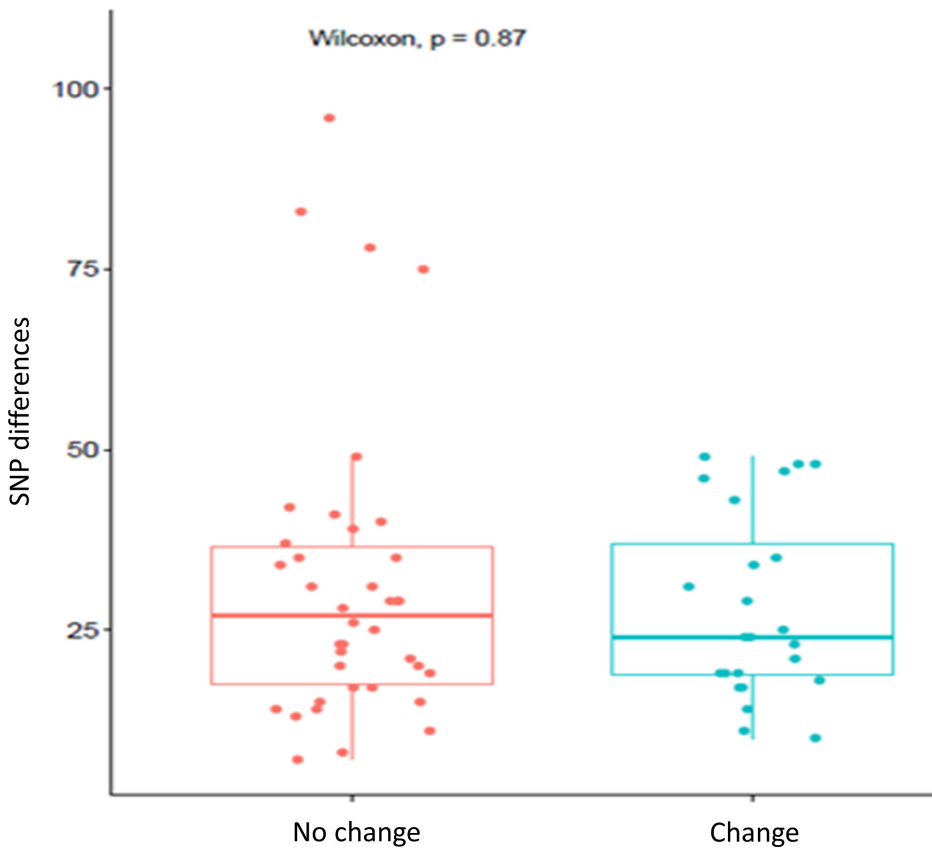


FIG 5 Left: SNP differences for each pairwise comparison among isolates from the same patient, where the resistant pattern did not change (S->S and R->R). Right: SNP differences for each pairwise comparison among isolates from the same patient where the resistant pattern changed (S->R). Boxplot R version 4.0.3

TABLE 2 SR pattern azoles^a

| ID | Patient ID | Date collected | Flu MIC value | Mutations PDR1 | Treatment ^f |
|-----------------------------|------------|----------------|---------------|---------------------------|------------------------|
| CAS10-2614_S20 | 22 | 27-Oct-10 | 8 | | Mica ^b |
| CAS12-3684_S68 | 22 | 21-Nov-11 | 4 | | Mica |
| CAS13-4530_S71 | 22 | 1-Nov-12 | 16 | | Mica; Flu; Ampho |
| CAS13-4778_S42 ^g | 22 | 13-Mar-13 | | | Mica; Flu; Ampho |
| CAS13-5011_S55 | 22 | 16-Jun-13 | 64 | p.Glu714Asp ^d | Mica; Flu |
| CAS08_0124_S32 | 1 | 28-May-08 | 32 | | |
| CAS12-4416_S11 | 1 | 3-Nov-12 | 64 | | Flu; Mica |
| CAS12-4158_S70 | 21 | 7-Jul-12 | 4 | | None |
| CAS13-4494_S47 | 21 | 19-Nov-12 | 8 | | Flu |
| CAS14-5293_S38 | 21 | 24-Nov-13 | 64 | | Mica; Flu |
| CAS14-5468_S77 | 21 | 10-Feb-14 | 32 | | Mica; Flu |
| CAS11-3025_S92 | 8 | 17-Mar-11 | 8 | | Mica |
| CAS12-3743_S58 | 8 | 3-Jan-12 | 128 | p.Gly1079Glu ^f | Flu; Mica |
| CAS10-2486_S79 | 15 | 28-Aug-10 | 4 | | Flu |
| CAS10-2596_S8 | 15 | 17-Oct-10 | 64 | p.Gly1099Ser | Flu; Mica |
| CAS11-3232_S57 | 15 | 16-May-11 | 8 | | Mica |
| CAS09-1541_S6 | 18 | 16-Sep-09 | 8 | | Unknown |
| CAS10-2227_S31 | 18 | 26-May-10 | 64 | p.Asn1091Asp ^d | Unknown |
| CAS09-1361_S41 | 19 | 19-Jul-09 | 8 | | Unknown |
| CAS09-1513_S89 | 19 | 27-Aug-09 | 256 | p.Arg376Gln ^e | Unknown |

^aShaded MIC values represent resistant isolates. Patient IDs in bold font represent patients in common with SR pattern for echinocandins.

^bMica = micafungin, Flu = fluconazole, Ampho = amphotericin B.

^cPatient received systemic antifungal medication to treat candidemia on or after positive culture date.

^dPreviously reported by Won E. et al 2021.

^eChange in the same position R376W previously reported by Ferrari et al 2009 (43).

^fChange in the same position G1079R previously reported by Won E. et al 2021.

^gFluconazole MIC value missing for this isolate.

antifungal of an unknown drug class. All three cases with acquired MDR were treated with both fluconazole and micafungin (Tables 1 and 2).

DISCUSSION

We analyzed serial isolates of *C. glabrata* from patients with multiple episodes of candidemia and used genomic and epidemiological data to assess the genetic diversity among these isolates. We showed that in most cases, multiple isolates from the same patient were genetically related and could be clearly distinguished from isolates obtained from other patients. We also identified known mutations in *FKS* and *PDR1* genes associated with acquired resistance to echinocandins and fluconazole, respectively. We found that isolates with different antifungal susceptibility profiles may be part of the same clonal population of *C. glabrata*.

The median SNP difference between isolates from the same patient was 30 SNPs, ranging from 7–96 SNPs. These SNP differences are similar to those reported in other studies that quantified the intra-host diversity of *C. glabrata* isolates using WGS. For example, Helmstetter et al. (28) detected 64–140 SNPs and insertions or deletions (Indels) between pairs of related strains (28). In addition, several studies compared the relatedness among *C. glabrata* isolates from the same patient using other molecular techniques (29, 30, 44, 45), and in general, they found similarity among isolates. Our findings are consistent with those reported by others and suggest that most patients are colonized by clonal populations of *C. glabrata* that contribute endogenous isolates to infection (45). However, several patients with heterogenous populations of unrelated isolates were identified.

Understanding how isolates from the same patient are related is essential for understanding nosocomial transmission and investigating outbreaks. Previous studies on *C. auris* showed that the SNP difference observed between isolates collected from the same patient can be used as a reference to assess transmission (46). However, these

distances are likely to differ for different species and depend on the genome's mutation rate and stability. The hypervariability of *C. glabrata* genomes has been shown previously (47). Here, we propose to use an upper boundary of 96 SNPs for defining genetically related isolates of *C. glabrata*. This SNP number is an approximation and may vary if new isolates are added. Although *C. glabrata* is not known to cause widespread nosocomial outbreaks, several cases of nosocomial acquisition (48, 49) and donor-to-host transmission of this pathogen have been reported (50, 51). We propose that the observed SNP difference within a patient could be used as a point of reference to assess donor-to-host transmission or nosocomial acquisition of *C. glabrata*.

In our study, 82% of patients were infected with isolates that were more closely related to each other than to isolates from other patients (7–96 SNPs), which suggests that these infections most likely originated from the endogenous populations of *C. glabrata* that are associated with each patient. However, six (18%) patients had infections with two or more genetically distinct strains (70,294–76,707 SNPs; Fig. S2). Previous studies based on MLST reported unrelated isolates from the same patient (2, 52). The most likely explanation for detecting isolates with different genotypes is that these patients were colonized by two or more genetically distinct populations of isolates. However, a secondary nosocomial infection with an exogenous strain cannot be ruled out (45). Detailed epidemiological data that were unavailable in our study are needed to distinguish between these two hypotheses.

To better understand the emergence of resistance and the relationships between resistant and susceptible isolates within the same patient, we investigated genetic substitutions in genes linked to resistance. Twenty echinocandin-resistant isolates had non-synonymous mutations in *FKS* genes (25% in *FKS1*; 75% in *FKS2*). The most common mutation observed in this study was the *FKS2* gene in position Ser-663 (53). We did not find mutations in HS2 of either *FKS1* or *FKS2* (8, 16, 24). The main fluconazole resistance mechanism in *C. glabrata* is upregulation of the expression of the ABC-transporter genes. Although assessing gene expression levels was outside the scope of our study, we screened the fluconazole-resistant isolates for non-synonymous changes in the sequence of the gene *PDR1*, which serves as the main transcription factor that modulates the gene expression of the ABC transporter (13), and we found nine different polymorphisms. Three of the amino acid changes that we found in *PDR1* (E714D, N109D, and R376Q) had been previously reported (12). Three patients had isolates with the corresponding mutations that were resistant to both echinocandins and fluconazole, suggesting *in vivo* acquisition of resistance to both fluconazole and echinocandins in response to treatment.

Our study demonstrated that isolates from the same patient were genetically related, independent of changes in susceptibility to antifungal drugs. Our data show that the acquisition of resistance to an antifungal drug did not have a significant impact on the SNP distances between serial isolates that remained susceptible or resistant over time compared with SNP distances between serial isolates that acquired resistance, confirming that the resistant phenotype could be achieved with very few genetic changes (26). A further implication of this finding is that the proposed boundary for defining genetically related isolates of *C. glabrata* is not affected by the susceptibility profile. Additional analysis of the number of SNP differences versus the time between isolate collection (Fig. S3) confirmed that the isolates were randomly pulled from the *C. glabrata* population within the host. Therefore, the observed genetic diversity reflects the inherent diversity in the population rather than accumulation of changes over time.

Our study has several limitations. First, SNP cutoffs are difficult to compare with those found in other studies since differences in bioinformatic methods and the selected reference genome can impact the SNP count. Second, our study included candidemia isolates only. To better define the diversity of isolates within patients, commensal isolates from patients' natural microbiomes should also be included. Third, since only a single isolate was recovered at any given timepoint, the diversity defined here likely does not represent the entire pooled *C. glabrata* population within the patient. Future analyses

using colonization isolates from the same patient may help validate these results further. Additionally, the SNP variation described may be influenced by several factors. Avenues for future research include the effect of the MLST sequence type (STs) of the *C. glabrata* isolates in the observed SNP diversity (27) and the presence of mutation in *MSH2* genes that lead to a hyper-mutable phenotype (54).

In conclusion, the comparison of serial isolates of *C. glabrata* from the same patient at different times during the course of an infection allowed estimating the expected intra-host diversity. It can be instrumental for investigating 1) cases of donor-to-host transmission, 2) nosocomial acquisition, and 3) microevolutionary processes leading to the emergence of drug resistance in *C. glabrata*. One of the main contributions of this work was the sequencing of 82 genomes of clinical isolates of *C. glabrata*. The WGS data were submitted to the NCBI (BioProject accession [PRJNA1009065](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1009065)) and together with MIC value susceptibility data (Table S2) published here could be included in future studies aimed at identifying new mechanisms of resistance in *C. glabrata* and new pathways of antifungal resistance evolution. In addition, we present here the use of an existing bioinformatics tool, SnpEff, for identifying mutations in human pathogenic fungi to facilitate further studies of genomic epidemiology of emerging resistance to antifungal drugs. WGS has become an essential part of epidemiological research to differentiate outbreak-associated illnesses from unrelated illnesses occurring at the same time. Our study provides a genomic framework for these future investigations.

ACKNOWLEDGMENTS

We thank the MDB laboratory team for continuing in providing support to perform all the fungal susceptibility testing procedures as part of our routine Emerging Infections Program (EIP) candidemia surveillance. We give special thanks to CDC's Office of Advanced Molecular Detection (OAMD). The authors acknowledge the support of Lindsay Bonner of the Maryland Emerging Infections Program; Alexia Zhang and Abiele Ariane Ebuon of the Oregon Emerging Infections Program; Caroline Graber, Sherry Hillis and Sandra Hardin of the Tennessee Emerging Infections Program; and the participating Emerging Infections Program Laboratories.

We acknowledge Oak Ridge Institute for Science and Education (ORISE) for financial support for EM.

AUTHOR AFFILIATIONS

¹Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

²ASRT, Inc., Atlanta, Georgia, USA

³Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

⁴Vanderbilt University Medical Center, Nashville, Tennessee, USA

⁵Oregon Public Health Division, Oregon Health Authority, Portland, USA

⁶Emory University School of Medicine, Decatur, Georgia, USA

AUTHOR ORCID^s

E. Misas  <http://orcid.org/0000-0001-6243-7716>

S. R. Lockhart  <http://orcid.org/0000-0002-4383-5994>

A. P. Litvintseva  <http://orcid.org/0000-0003-1086-8041>

FUNDING

| Funder | Grant(s) | Author(s) |
|----------------------------|----------|-----------|
| ORAU/ORISE | | E. Misas |

ADDITIONAL FILES

The following material is available [online](#).

Supplemental Material

Supplementary Figure S1 (JCM01140-23-S0001.tif). Neighbor-joining tree with 1000 bootstrap replicates.

Supplementary Figure S2 (JCM01140-23-S0002.tif). Neighbor-joining phylogeny of *C. glabrata*, patient with isolates that exhibited at least two unrelated isolates.

Supplementary Figure S3 (JCM01140-23-S0003.tif). Number of SNPs between pairs of isolates from the same patient vs. number of days between collection samples.

Supplementary Figure S4 (JCM01140-23-S0004.tif). Number of variant effects of each by functional class and by type and region from SnpEff report.

Supplemental material (JCM01140-23-S0005.docx). Supplementary tables and supplementary figure legends.

REFERENCES

- Barantsevich N, Barantsevich E. 2022. Diagnosis and treatment of invasive candidiasis. *Antibiotics (Basel)* 11:718. <https://doi.org/10.3390/antibiotics11060718>
- Jensen RH, Johansen HK, Søres LM, Lemming LE, Rosenvinge FS, Nielsen L, Olesen B, Kristensen L, Dzajic E, Astvad KMT, Arendrup MC. 2015. Posttreatment antifungal resistance among colonizing candida isolates in candidemia patients: results from a systematic multicenter study. *Antimicrob Agents Chemother* 60:1500–1508. <https://doi.org/10.1128/AAC.01763-15>
- Arastehfar A, Daneshnia F, Salehi M, Yaşar M, Hoşbul T, Ilkit M, Pan W, Hagen F, Arslan N, Türk-Dağlı H, Hilmioglu-Polat S, Perlin DS, Lass-Flörl C. 2020. Low level of antifungal resistance of *Candida glabrata* blood isolates in Turkey: fluconazole minimum inhibitory concentration and *FKS* mutations can predict therapeutic failure. *Mycoses* 63:911–920. <https://doi.org/10.1111/myc.13104>
- Pfaller MA, Andes DR, Diekema DJ, Horn DL, Reboli AC, Rotstein C, Franks B, Azie NE. 2014. Epidemiology and outcomes of invasive candidiasis due to non-albicans species of candida in 2,496 patients: data from the prospective antifungal therapy (PATH) registry 2004–2008. *PLoS One* 9:e101510. <https://doi.org/10.1371/journal.pone.0101510>
- Berkow EL, Lockhart SR. 2017. Fluconazole resistance in *Candida* species: a current perspective. *Infect Drug Resist* 10:237–245. <https://doi.org/10.2147/IDR.S118892>
- Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. 2019. Twenty years of the SENTRY antifungal surveillance program: results for *Candida* species from 1997–2016. *Open Forum Infect Dis* 6:579–594. <https://doi.org/10.1093/ofid/ofy358>
- Rodrigues CF, Silva S, Henriques M. 2014. *Candida glabrata*: a review of its features and resistance. *Eur J Clin Microbiol Infect Dis* 33:673–688. <https://doi.org/10.1007/s10096-013-2009-3>
- Zimbeck AJ, Iqbal N, Ahlquist AM, Farley MM, Harrison LH, Chiller T, Lockhart SR. 2010. *FKS* mutations and elevated echinocandin MIC values among *Candida glabrata* isolates from U.S. population-based surveillance. *Antimicrob Agents Chemother* 54:5042–5047. <https://doi.org/10.1128/AAC.00836-10>
- Pappas PG, Kauffman CA, Andes D, Benjamin DK, Calandra TF, Edwards JE, Filler SG, Fisher JF, Kullberg B-J, Ostrosky-Zeichner L, Reboli AC, Rex JH, Walsh TJ, Sobel JD. 2009. Clinical practice guidelines for the management of candidiasis: 2009 update by the infectious diseases society of America. *Clin Infect Dis* 48:503–535. <https://doi.org/10.1086/596757>
- Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH, Sobel JD, Pappas PG, Kullberg BJ. 2012. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. *Clin Infect Dis* 54:1110–1122. <https://doi.org/10.1093/cid/cis021>
- Nivoix Y, Ledoux MP, Herbrecht R. 2020. Antifungal therapy: new and evolving therapies. *Semin Respir Crit Care Med* 41:158–174. <https://doi.org/10.1055/s-0039-3400291>
- Won EJ, Choi MJ, Kim M-N, Yong D, Lee WG, Uh Y, Kim TS, Byeon SA, Lee SY, Kim SH, Shin JH. 2021. Fluconazole-resistant *Candida glabrata* bloodstream isolates, South Korea, 2008–2018. *Emerg Infect Dis* 27:779–788. <https://doi.org/10.3201/eid2703.203482>
- Vermitsky J-P, Edlind TD. 2004. Azole resistance in *Candida glabrata*: coordinate upregulation of multidrug transporters and evidence for a *PDR1*-like transcription factor. *Antimicrob Agents Chemother* 48:3773–3781. <https://doi.org/10.1128/AAC.48.10.3773-3781.2004>
- Paul S, McDonald WH, Moye-Rowley WS. 2018. Negative regulation of *Candida glabrata PDR1* by the deubiquitinase subunit Bre5 occurs in a ubiquitin independent manner. *Mol Microbiol* 110:309–323. <https://doi.org/10.1111/mmi.14109>
- Pais P, Galocha M, Viana R, Cavalheiro M, Pereira D, Teixeira MC. 2019. Microevolution of the pathogenic yeasts *Candida albicans* and *Candida glabrata* during antifungal therapy and host infection. *Microb Cell* 6:142–159. <https://doi.org/10.15698/mic2019.03.670>
- Katiyar SK, Alastruey-Izquierdo A, Healey KR, Johnson ME, Perlin DS, Edlind TD. 2012. *FKS1* and *FKS2* are functionally redundant but differentially regulated in *Candida glabrata*: implications for echinocandin resistance. *Antimicrob Agents Chemother* 56:6304–6309. <https://doi.org/10.1128/AAC.00813-12>
- Perlin DS. 2018. MSGERC Biennial Meeting 2018: antifungal resistance among *Candida* Spp. Available from: https://msgerc.org/resources/Documents/2018%20Speaker%20Handouts/Candida_2_Perlin.pdf
- Cleary JD, Garcia-Effron G, Chapman SW, Perlin DS. 2008. Reduced *Candida glabrata* susceptibility secondary to an *FKS1* mutation developed during candidemia treatment. *Antimicrob Agents Chemother* 52:2263–2265. <https://doi.org/10.1128/AAC.01568-07>
- Thompson GR, Wiederhold NP, Vallor AC, Villareal NC, Lewis JS, Patterson TF. 2008. Development of caspofungin resistance following prolonged therapy for invasive candidiasis secondary to *Candida glabrata* infection. *Antimicrob Agents Chemother* 52:3783–3785. <https://doi.org/10.1128/AAC.00473-08>
- Costa-de-Oliveira S, Marcos Miranda I, Silva RM, Pinto E Silva A, Rocha R, Amorim A, Gonçalves Rodrigues A, Pina-Vaz C. 2011. *FKS2* mutations associated with decreased echinocandin susceptibility of *Candida glabrata* following anidulafungin therapy. *Antimicrob Agents Chemother* 55:1312–1314. <https://doi.org/10.1128/AAC.00589-10>
- Bordallo-Cardona MÁ, Sánchez-Carrillo C, Bouza E, Muñoz P, Escibano P, Guinea J. 2019. Detection of echinocandin-resistant *Candida glabrata* in blood cultures spiked with different percentages of *FKS2* mutants. *Antimicrob Agents Chemother* 63:e02004-18. <https://doi.org/10.1128/AAC.02004-18>
- Chapeland-Leclerc F, Hennequin C, Papon N, Noël T, Girard A, Socié G, Ribaud P, Lacroix C. 2010. Acquisition of flucytosine, azole, and caspofungin resistance in *Candida glabrata* bloodstream isolates serially obtained from a hematopoietic stem cell transplant recipient. *Antimicrob Agents Chemother* 54:1360–1362. <https://doi.org/10.1128/AAC.01138-09>

23. Borst A, Raimer MT, Warnock DW, Morrison CJ, Arthington-Skaggs BA. 2005. Rapid acquisition of stable azole resistance by *Candida glabrata* isolates obtained before the clinical introduction of fluconazole. *Antimicrob Agents Chemother* 49:783–787. <https://doi.org/10.1128/AAC.49.2.783-787.2005>
24. Garcia-Effron G, Lee S, Park S, Cleary JD, Perlin DS. 2009. Effect of *Candida glabrata* *FKS1* and *FKS2* mutations on echinocandin sensitivity and kinetics of 1,3-beta-D-glucan synthase: implication for the existing susceptibility breakpoint. *Antimicrob Agents Chemother* 53:3690–3699. <https://doi.org/10.1128/AAC.00443-09>
25. Corcione S, D'Avolio A, Pasero D, Trentalange A, Pagani N, Sanguinetti M, De Rosa FG. 2019. Acquisition of *FKS2* mutation after echinocandin treatment of infective endocarditis by *Candida glabrata*. *Infez Med* 27:328–331.
26. Ksiezopolska E, Schikora-Tamarit MA, Beyer R, Nunez-Rodriguez JC, Schüller C, Gabaldón T. 2021. Narrow mutational signatures drive acquisition of multidrug resistance in the fungal pathogen *Candida glabrata*. *Curr Biol* 31:5314–5326. <https://doi.org/10.1016/j.cub.2021.09.084>
27. Dodgson AR, Pujol C, Denning DW, Soll DR, Fox AJ. 2003. Multilocus sequence typing of *Candida glabrata* reveals geographically enriched clades. *J Clin Microbiol* 41:5709–5717. <https://doi.org/10.1128/JCM.41.12.5709-5717.2003>
28. Helmstetter N, Chybowska AD, Delaney C, Da Silva Dantas A, Gifford H, Wacker T, Munro C, Warris A, Jones B, Cuomo CA, Wilson D, Ramage G, Farrer RA. 2022. Population genetics and microevolution of clinical *Candida glabrata* reveals recombinant sequence types and hyper-variation within mitochondrial genomes, virulence genes, and drug targets. *Genetics* 221:iyac031. <https://doi.org/10.1093/genetics/iyac031>
29. Barber AE, Weber M, Kaerger K, Linde J, Gölz H, Duerschmied D, Markert A, Guthke R, Walther G, Kurzai O. 2019. Comparative genomics of serial *Candida glabrata* isolates and the rapid acquisition of echinocandin resistance during therapy. *Antimicrob Agents Chemother* 63:e01628-18. <https://doi.org/10.1128/AAC.01628-18>
30. Vale-Silva L, Beaudouin E, Tran VDT, Sanglard D. 2017. Comparative genomics of two sequential *Candida glabrata* clinical isolates. *G3 (Bethesda)* 7:2413–2426. <https://doi.org/10.1534/g3.117.042887>
31. Clinical and Laboratory Standard Institute, W., PA. 2017. Reference method for broth dilution antifungal susceptibility testing of Yeast-fourth edition, CLSI document M27-A4
32. Bagal UR, Phan J, Welsh RM, Misas E, Wagner D, Gade L, Litvintseva AP, Cuomo CA, Chow NA. 2022. MycoSNP: a portable workflow for performing whole-genome sequencing analysis of *Candida auris*. *Methods Mol Biol* 2517:215–228. https://doi.org/10.1007/978-1-0716-2417-3_17
33. Delcher AL, Salzberg SL, Phillippy AM. 2003. Using mummer to identify similar regions in large sequence SETS. *Curr Protoc Bioinformatics* Chapter 10:Unit <https://doi.org/10.1002/0471250953.bi1003s00>
34. Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26:841–842. <https://doi.org/10.1093/bioinformatics/btq033>
35. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
36. Lo C-C, Chain PSG. 2014. Rapid evaluation and quality control of next generation sequencing data with FaQCs. *BMC Bioinformatics* 15:366. <https://doi.org/10.1186/s12859-014-0366-2>
37. Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM
38. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The genome analysis toolkit: a mapreduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 20:1297–1303. <https://doi.org/10.1101/gr.107524.110>
39. Kumar S, Stecher G, Li M, Knyaz C, Tamura K, Battistuzzi FU. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547–1549. <https://doi.org/10.1093/molbev/msy096>
40. Argimón S, Abudahab K, Goater RJE, Fedosejev A, Bhai J, Glasner C, Feil EJ, Holden MTG, Yeats CA, Grundmann H, Spratt BG, Aanensen DM. 2016. Microreact: visualizing and sharing data for genomic epidemiology and phylogeography. *Microb Genom* 2:e000093. <https://doi.org/10.1099/mgen.0.000093>
41. R_Core_Team. 2021. R: a language and environment for statistical computing
42. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly (Austin)* 6:80–92. <https://doi.org/10.4161/fly.19695>
43. Ferrari S, Ischer F, Calabrese D, Posteraro B, Sanguinetti M, Fadda G, Rohde B, Bauser C, Bader O, Sanglard D. 2009. Gain of function mutations in CgPDR1 of *Candida glabrata* not only mediate antifungal resistance but also enhance virulence. *PLoS Pathog* 5:e1000268. <https://doi.org/10.1371/journal.ppat.1000268>
44. Singh-Babak SD, Babak T, Diezmann S, Hill JA, Xie JL, Chen Y-L, Poutanen SM, Rennie RP, Heitman J, Cowen LE. 2012. Global analysis of the evolution and mechanism of echinocandin resistance in *Candida glabrata*. *PLoS Pathog* 8:e1002718. <https://doi.org/10.1371/journal.ppat.1002718>
45. Carreté L, Ksiezopolska E, Gómez-Molero E, Angoulvant A, Bader O, Fairhead C, Gabaldón T. 2019. Genome comparisons of *Candida glabrata* serial clinical isolates reveal patterns of genetic variation in infecting clonal populations. *Front Microbiol* 10:112. <https://doi.org/10.3389/fmicb.2019.00112>
46. Chow NA, Gade L, Tsay SV, Forsberg K, Greenko JA, Southwick KL, Barrett PM, Kerins JL, Lockhart SR, Chiller TM, Litvintseva AP. 2018. Multiple introductions and subsequent transmission of multidrug-resistant *Candida auris* in the USA: a molecular epidemiological survey. *Lancet Infect Dis* 18:1377–1384. [https://doi.org/10.1016/S1473-3099\(18\)30597-8](https://doi.org/10.1016/S1473-3099(18)30597-8)
47. Ahmad KM, Kokošar J, Guo X, Gu Z, Ishchuk OP, Piškur J. 2014. Genome structure and dynamics of the yeast pathogen *Candida glabrata*. *FEMS Yeast Res* 14:529–535. <https://doi.org/10.1111/1567-1364.12145>
48. Vazquez JA, Dembry LM, Sanchez V, Vazquez MA, Sobel JD, Dmouchowski C, Zervos MJ. 1998. Nosocomial *Candida glabrata* colonization: an epidemiologic study. *J Clin Microbiol* 36:421–426. <https://doi.org/10.1128/JCM.36.2.421-426.1998>
49. Paluchowska P, Tokarczyk M, Bogusz B, Skiba I, Budak A. 2014. Molecular epidemiology of *Candida albicans* and *Candida glabrata* strains isolated from intensive care unit patients in Poland. *Mem Inst Oswaldo Cruz* 109:436–441. <https://doi.org/10.1590/0074-0276140099>
50. Tappeiner C, Goldblum D, Zimmerli S, Fux C, Frueh BE. 2009. Donor-to-host transmission of *Candida glabrata* to both recipients of corneal transplants from the same donor. *Cornea* 28:228–230. <https://doi.org/10.1097/ICO.0b013e318183a3e3>
51. Al-Assiri A, Al-Jastaneiah S, Al-Khalaf A, Al-Fraikh H, Wagoner MD. 2006. Late-onset donor-to-host transmission of *Candida glabrata* following corneal transplantation. *Cornea* 25:123–125. <https://doi.org/10.1097/O1.ico.0000164777.80879.07>
52. Odds FC, Hanson MF, Davidson AD, Jacobsen MD, Wright P, Whyte JA, Gow NAR, Jones BL. 2007. One year prospective survey of candida bloodstream infections in Scotland. *J Med Microbiol* 56:1066–1075. <https://doi.org/10.1099/jmm.0.47239-0>
53. Aldejohann AMet al. 2021. Emergence of resistant *Candida glabrata* in Germany. *JAC Antimicrob Resist* 3. <https://doi.org/10.1093/jacamr/dlab122>
54. Healey KR, Zhao Y, Perez WB, Lockhart SR, Sobel JD, Farmakiotis D, Kontoyiannis DP, Sanglard D, Taj-Aldeen SJ, Alexander BD, Jimenez-Ortigosa C, Shor E, Perlin DS. 2016. Prevalent mutator genotype identified in fungal pathogen *Candida glabrata* promotes multi-drug resistance. *Nat Commun* 7:11128. <https://doi.org/10.1038/ncomms11128>