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Azole resistance in *Candida glabrata* clinical isolates from global surveillance is associated with efflux overexpression

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ABSTRACT

Objectives: We evaluated the azole resistance mechanisms and epidemiology of fluconazole-resistant *Candida glabrata* from a global survey.
Methods: A total of 2992 *Candida* spp. isolates collected during 2018–2019 were susceptibility tested by the broth microdilution reference method following CLSI guidelines. Fluconazole-resistant *C. glabrata* isolates were submitted to whole genome sequencing and gene expression assays using qRT-PCR.
Results: Among 561 CGLA isolates tested, 34 (6.1%) were fluconazole resistant. These isolates were collected from 11 countries and mainly recovered from bloodstream infections (79.4%). All fluconazole-resistant *C. glabrata* isolates were non-wild type for voriconazole, 24/34 were non-wild type for posaconazole, but only 2/34 were non-wild type for itraconazole. Isavuconazole MIC values ranged from 0.25 to >4 mg/L. Fluconazole-resistant *C. glabrata* isolates belonged to 14 different sequence types (ST). None of the isolates exhibited alterations in ERG3 or ERG11, the target of azoles. All but two fluconazole-resistant isolates displayed overexpression of CgCDR1 (22/34; 64.7%) and/or CgCDR2 (26/34; 76.5%), while 16 isolates had both genes overexpressed. Overexpression of CgSNQ2 or ERG11 was not observed. Gain of function (GoF) alterations in the transcription factor CgPDR1 were noted in 14 isolates. Four (11.8%) isolates that were nonsusceptible to one or more echinocandins had FKS2 HS1 alterations (2 S663P and 2 F659Y/deletion).
Conclusion: Fluconazole-resistant *C. glabrata* was driven by overexpression of CgCDR1 and/or CgCDR2. GoF alterations in PDR1 that have been associated with increased virulence were observed. Susceptibility results and surveillance data are needed to guide treatment for these isolates.

1. Introduction

Candida glabrata, recently renamed *Nakaseomyces glabrataa* [1], is an important cause of human infections [2]. According to a 20-year global survey of invasive fungal infections, *C. glabrata* isolates increased in occurrence over time, ranking as the second most common *Candida* species isolated in Asia-Pacific, Europe, and North America by the end of the study period [3]. *C. glabrata* isolates are a threat to human health due to their ability to develop resistance to antifungal agents [4]. Unlike other *Candida* species that are diploid and usually require alterations in both gene alleles to confer resistance, *C. glabrata* is a haploid organism [5,6], and resistance can be conferred by a single amino acid alteration that facilitates the development of antifungal resistance and the accumulation of mutations, leading to multidrug resistance (MDR) [6].

Azoles, echinocandins, polyenes, and the pyrimidine analogue flucytosine are active against *C. glabrata* isolates. However, resistance to azoles and echinocandins is reported in this species. Fluconazole resistance has been reported to be as high as 10% among *C. glabrata* clinical isolates [3,4,7]. Echinocandin resistance in this species can be up to 5%, depending on the region. Organisms resistant to these two widely used antifungal classes have been described and are categorised as MDR to two antifungal classes. In a study by Alexander et al. [8], the authors reported that resistance to echinocandins among fluconazole-resistant isolates was as high as 14.1% among *C. glabrata* isolates collected from bloodstream infections during 10 years in a single U.S. hospital. Another U.S.-based analysis identified that fluconazole resistance was 8.6%

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among *C. glabrata* isolates from four hospitals, and 1.3% of these isolates were MDR [9].

Resistance to azoles in *C. glabrata* can arise during therapy due to induction of overexpression of efflux pumps. The increased expression of the ATP-binding cassette (ABC) transporters CgCDR1, CgCDR2 (also known as PDH1), or CgSNQ2 are the main resistance mechanism reported among clinical isolates [10–12]. The upregulation of these genes occurs by gain of function (GoF) mutations on the transcriptional factor of pleiotropic drug resistance, PDR1 or CgPDR1 [13,14]. Furthermore, studies have shown that mitochondria as well as mutations previously identified in mutant libraries could have an impact in fluconazole resistance [15]. Alterations and overexpression of *ERG11* have been observed among *C. glabrata* isolates [16], but these mechanisms seem to be less common. Additionally, mutations in regulators such as UPC2 and TAQ1 that confer azole resistance in *Candida albicans* have not been deemed as relevant in *C. glabrata* [17]. As with other *Candida* species, resistance to echinocandin in *C. glabrata* is caused by alterations in the hotspot regions of the 1,3-b-glucan synthase [18].

An evaluation the SENTRY Antifungal Surveillance Program from 1996 to 2014 documented an increase in fluconazole-resistant *C. glabrata* isolates from 8.6% to 10.1% (3). In this study, we expanded this knowledge by evaluating the occurrence of fluconazole resistance among *C. glabrata* isolates collected in the SENTRY Program during 2018 and 2019. Additionally, we evaluated the echinocandin and MDR rates among the tested isolates and analysed the mechanisms of resistance and epidemiology of fluconazole-resistant isolates in this species.

2. Materials and Methods

A total of 561 *C. glabrata* isolates were collected among 2992 invasive fungal clinical isolates submitted to a global surveillance initiative during 2018 and 2019. The isolates were non-duplicated and consecutively collected in 68 hospitals located in 28 countries. Species identification was performed using matrix-assisted laser desorption ionization time of flight mass spectrometry or molecular methods if an acceptable identification was not achieved, as described previously [19,20].

Susceptibility testing was performed by using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution reference method (21). CLSI clinical breakpoints were used where available [21]. Published epidemiologic cut-off values (ECVs) were applied when breakpoints were not available [22]. Quality control was performed as recommended in the CLSI document M60 [23] using *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019. All results were within established ranges.

C. glabrata isolates displaying resistance to fluconazole were submitted to whole genome sequencing (WGS) on a MiSeq Sequencer (Illumina, San Diego, CA, USA). Total genomic DNA was used as input material for library construction prepared using the Nextera XT™ library construction protocol and index kit (Illumina, San Diego, CA, USA) following manufacturer instructions. Reads were quality trimmed using Sickle [24] and error corrected using BayesHammer [25]. Each sample was assembled using a reference-guided assembly in DNASTAR SeqMan NGen v.14.0 (Madison, WI, USA). Sequences of *FKS1* and *FKS2* hotspots were compared to echinocandin-susceptible isolates, as previously described [18]. *ERG11, CgCDR1, CgCDR2*, and *CgSNQ2* sequences were analysed and compared to those of *C. glabrata* ATCC 60032 and *C. glabrata* ATCC 93330. Multilocus sequence typing (MLST) was performed using WGS data and the MLST database (PubMLST) available at https://pubmlst.org [26].

The expression levels of *ERG11, CgCDR1, CgCDR2*, and *CgSNQ2* were determined by quantitative real-time PCR (qRT-PCR) using high-quality DNA-free RNA preparations. Total RNA was extracted from 2×10^7 mid-log-phase yeast cells grown in Sabouraud liquid medium (cell density at OD_{600} of 0.25–0.3). Cells were harvested, suspended in 2 mL freshly prepared Y1 buffer containing 0.1% βME and 150 units lyticase, and incubated for 10–30 minutes at 30°C with gentle shaking to generate spheroplasts. RNA was extracted from the spheroplasts using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Residual DNA was eliminated with RNase-free DNase (Promega, Wisconsin, USA). mRNA was quantified, and the sample quality was assessed using the RNA 6000 Pico kit on the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), according to manufacturer instructions. Only preparations with an RNA integrity number >6.5 that showed no visual degradation were used for experiments. Relative quantification of target genes was performed in triplicate by normalization to an endogenous reference gene (*ACT1*) on the StepOne Plus instrument (Life Technologies, Carlsbad, CA, USA) using custom-designed primers showing ≥90.0% efficiency. Transcription levels were considered significantly different if a 5-fold difference was noted compared with *C. glabrata* ATCC 60032.

3. Results

Fluconazole resistance was observed among 6.1% of the 561 *C. glabrata* isolates collected worldwide (Table 1). These rates varied by region and were highest in North America (8.1%), followed by Europe 5.9% (Fig. 1). Only 2.7% of the 73 *C. glabrata* isolates from Asia-Pacific countries were resistant to fluconazole. None of these isolates were noted in Latin American countries (Fig. 1). All fluconazole-resistant *C. glabrata* isolates were non-wild type for voriconazole, 24/34 were non-wild type to posaconazole, but only two were non-wild type to itraconazole. Isavuconazole MIC values ranged from 0.25 to >4 mg/L (Table 2).

Overall, 2.3% (13/561) isolates were resistant to at least one of the echinocandins tested—anidulafungin, caspofungin, or micafungin. Resistance to any of these echinocandins alone was noted among 2.1% of the overall collection of *C. glabrata* (Table 2). One isolate had an elevated MIC to micafungin only. Four (11.8%) isolates that were nonsusceptible to one or more echinocandins had FKS2 HS1 alterations: two isolates each had S663P and F659Y/deletions (Table 2).

Only three (8.8%) fluconazole-resistant isolates were also resistant to the echinocandins (Table 2). The isolates resistant to both antifungal classes were recovered from bloodstream infections in patients hospitalised in Slovenia and in Ohio and Colorado in the United States.

Sixteen (16/34; 47.1%) of the fluconazole-resistant *C. glabrata* isolates were recovered in U.S. hospitals. These institutions were located in 12 states, with 1 to 3 isolates each per state. The remaining 18 isolates were recovered in 10 countries: Spain (5 isolates), Slovenia (3), Germany (2), Korea (2), Belgium (1), Canada (1), Czech Republic (1), Ireland (1), Sweden (1), and the United Kingdom (1).

Fluconazole-resistant *C. glabrata* belonged to 14 sequence types (STs; Table 2). The most common ST was ST3 ($n = 7$) observed in Spain and the United States. ST7 or the single locus variant ST200 ($n = 5$) was noted in five countries. ST6 isolates ($n = 4$) were noted in Slovenia and Spain. All fluconazole-resistant *C. glabrata* isolates from Spain were identified in the same hospital. These isolates belonged to two STs, suggesting both the emergence of resistance in multiple instances and clonal dissemination of the resistant lineages.

All 34 fluconazole-resistant *C. glabrata* isolates displayed wild type sequences for *ERG3* and *ERG11*, the target of azoles. CgCDR1 was overexpressed 10 × to 85 × among 20 fluconazole-resistant *C. glabrata* isolates (Table 2). Two isolates displayed a modest increase in the CgCDR1 expression (5 × and 8 × compared to the

Table 1
Activity of antifungal agents tested against 561 *Candida glabrata* using the CLSI broth microdilution method

Antifungal agent	MIC_{50}	MIC_{90}	CLSI[a] %S	%I	%R	ECV[a] %WT	%NWT
Fluconazole	4	16	93.9		6.1 [b]	88.9	11.1
Isavuconazole	0.06	0.5					
Itraconazole	0.5	1				99.6	0.4
Posaconazole	0.25	1				95.5	4.5
Voriconazole	0.06	0.5				89.8	10.2
Anidulafungin	0.03	0.12	96.4	1.4	2.1	97.9	2.1
Caspofungin	0.03	0.06	97.1	0.7	2.1		
Micafungin	0.015	0.03	97.7	0.2	2.1	96.1	3.9
Amphotericin B	1	1				100.0	0.0

[a] Criteria published by CLSI M60 [40] and M61 [41]. ECV criteria published in CLSI M59 [42].
[b] Non-resistant is interpreted as susceptible-dose dependent.

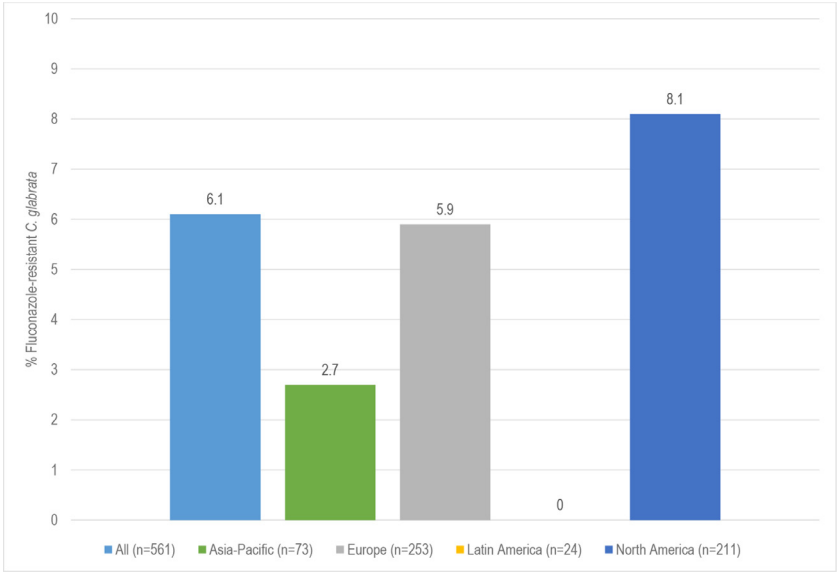

Fig. 1. Fluconazole resistance rates of *C. glabrata* from a global surveillance study.

control). CgCDR2 was overexpressed in 18 isolates (>10X), and 8 isolates had expression rates ranging from 5 × to 9 × compared to the control. Sixteen (47.1%) isolates exhibited a concomitant increased expression of CgCDR1 and CgCDR2. Overexpression of at least one efflux system was identified in all but two isolates: one from Korea and another from Spain. These isolates displayed fluconazole MIC values of 64 mg/L and voriconazole MIC values of 2 mg/L, but wild-type MIC values for other azoles. All isolates displayed basal expression of *CgSNQ2* and *ERG11*.

Gain of function (GoF) alterations in transcription factor PDR1 that were previously described in the literature were observed in 14 isolates (Table 1). Alterations in position 297 (W→L/C) were detected in three isolates, two from Spain and one from the United States, and amino acid substitutions in position 346 (G→D/S) were noted in one isolate from Slovenia and one from the United States. The 11 remaining isolates that displayed GoF mutations in PDR1 had the discrete alterations reported by Ferrari et al., but other mutations were also noted in the PDR1 sequences of fluconazole-resistant *C. glabrata* isolates.

The MSH2 mutator genotype (V239L) was noted in 10 isolates belonging to STs 2, 7, 8, 11, and 200; however, none of these isolates were MDR to the echinocandins and fluconazole concomitantly.

4. Discussion

According to the U.S. Centers for Disease Control and Prevention (CDC), MDR among *Candida* species is responsible for 34 800 infections and 1700 deaths in U.S. hospitals during 2017 [27]. In recent years, the nosocomial dissemination of *C. auris* isolates [28] resistant to one to three antifungal classes raised awareness of the emergence of MDR among the *Candida* species. Despite the emerging importance of *C. auris*, its prevalence as a cause of invasive infections is generally low [29]. In contrast, *C. glabrata* is the second most prevalent *Candida* species in various countries [2] and most regions of the word [3]; plus, this species has the highest MDR rates among the common *Candida* species. *C. glabrata* isolates display intrinsically higher MIC values for fluconazole ($MIC_{50/90}$, 4/16 mg/L) when compared to *C. albicans* ($MIC_{50/90}$, 0.015/0.03 mg/L) [30]. Additionally, acquired echinocandin resistance is higher in *C. glabrata* when compared to other *Candida* species [3,30].

In this study, we evaluated the fluconazole resistance rates in two recent years of a global surveillance program and noted that fluconazole resistance rates were as high as 8% in North America and almost 6% in Europe. Lower rates were observed in Asia-Pacific and Latin America. Alexander et al. reported that among *C. glabrata* isolates, the fluconazole resistance rates in a U.S. hospital was 30%

Table 2
Susceptibility profiles and genetic characteristics of fluconazole-resistant *C. glabrata*

Country	ST	Study Year	MIC (mg/L)[a]								Gene expression relative to control[b]		Sequence alterations[c]		
			Fluconazole	Isavuconazole	Itraconazole	Posaconazole	Voriconazole	Anidulafungin	Caspofungin	Micafungin	CDR1	CDR2	FKS2 HS1	MSH2	PDR1
Belgium	7	2018	**64**	2	2	[2]	[2]	0.12	0.06	[0.06]	0.95	**18.77**		V239L	V329A,**T885A**
Canada	19	2018	**128**	2	4	[2]	[4]	0.12	0.06	0.03	**51.56**	**56.47**			D554G
Czech Republic	45	2018	**64**	2	1	1	[1]	0.06	0.03	0.008	**15.77**	**18.12**			R250K,**L280F**
Germany	2	2018	**64**	1	2	[2]	[1]	0.03	0.03	0.015	**36.63**	**11.43**		A942T,V239L	A731E
Germany	177	2019	**128**	2	2	[2]	[4]	0.06	0.06	0.03	1.35	**32.97**			**G334W,**E492K,N496S
Ireland	7	2018	**64**	2	2	[2]	[2]	0.06	0.03	0.015	1.40	**7.91**		V239L	N928I
Korea	26	2018	**128**	2	4	[4]	[4]	0.06	0.06	0.03	**5.24**	**13.47**			**E555K**
Korea	200	2018	**64**	2	2	1	[2]	0.03	0.015	0.008	2.20	3.02		V239L	R956G
Slovenia	6	2018	**128**	2	4	[4]	[4]	0.06	0.06	0.03	1.66	**5.13**			**G346D**
Slovenia	6	2018	**128**	2	2	[2]	[2]	**1**	0.25	[0.06]	0.89	**8.58**	F659Y		
Slovenia	7	2018	**64**	4	[>4]	[4]	[4]	0.12	0.06	0.015	2.49	**29.76**		V239L	**T1080I**
Spain	3	2018	**64**	4	2	[2]	[2]	0.015	0.008	0.004	**32.34**	**27.58**			P76S,P143T,D243N,**W297L**
Spain	3	2019	**128**	4	4	[2]	[4]	0.03	0.03	0.015	**11.56**	2.59			P76S,P143T,D243N,L344S
Spain	3	2018	**128**	4	4	[2]	[4]	0.015	0.015	0.008	**42.28**	**21.38**			P76S,P143T,D243N,**W297L**
Spain	6	2019	**64**	2	1	1	[2]	0.03	0.03	0.015	0.32	4.81			K778E
Spain	6	2019	**128**	2	2	[2]	[2]	0.015	0.03	0.015	0.74	**12.68**		I720T	H576Y
Sweden	2	2019	**128**	2	1	1	[2]	0.03	0.06	0.03	**45.58**	**33.96**		A942T,V239L	E369D
United Kingdom	10	2019	**64**	2	2	[2]	[2]	0.015	0.03	0.03	**32.04**	4.32		P208S,N890I	**S942F**
USA	3	2019	**64**	2	1	0.5	[2]	[0.25]	0.25	**0.25**	**21.27**	**19.31**	F659 dele-tion		P76S,P143T,D243N
USA	3	2019	**64**	1	1	1	[1]	0.03	0.03	0.015	**8.69**	2.02			P76S,P143T,D243N,**G583S**
USA	3	2018	**128**	2	2	1	[2]	0.06	0.06	0.015	**22.14**	**9.24**			P76S,P143T,D243N,S337F
USA	3	2019	**128**	4	4	[2]	[4]	0.015	0.06	0.03	**27.52**	**10.65**			P76S,P143T,D243N,S391L
USA	7	2019	**64**	1	2	1	[1]	0.12	0.12	0.03	0.79	**5.31**		V239L	**S316I**
USA	8	2018	**64**	4	4	[2]	[4]	0.03	0.12	0.015	**19.77**	**49.01**		A942T,V239L	
USA	8	2019	**128**	2	2	[2]	[2]	0.12	0.03	0.015	**85.31**	**22.52**		A942T,V239L	D554G
USA	10	2019	128	4	4	[2]	[4]	0.06	0.03	0.03	**34.98**	**7.11**		P208S,N890I	S391L
USA	11	2019	128	2	[>4]	[2]	[4]	0.03	0.03	0.03	**36.19**	**11.08**		A942T,V239L	**G346S**
USA	16	2018	64	2	2	[2]	[2]	0.06	0.03	0.015	**31.66**	**8.09**		E231G,L269F	T292K
USA	16	2018	64	2	2	[2]	[2]	0.06	0.03	0.015	**32.53**	1.76		E231G,L269F	R761S
USA	16	2019	128	4	2	[2]	[4]	**1**	**0.5**	**0.5**	**14.98**	2.20	S663P	E231G,L269F	S216R
USA	19	2018	64	4	2	1	[2]	0.06	0.03	0.015	**21.66**	**11.25**			**W297C**
USA	19	2019	128	0.25	0.5	0.5	[0.5]	[0.25]	0.12	[0.12]	**10.63**	1.58	S663P		N1086Y
USA	26	2019	64	1	2	[2]	[2]	0.015	0.015	0.008	1.35	**11.92**			G611S,S1048F
USA	55	2019	64	0.5	1	[2]	[1]	0.12	0.06	0.03	0.93	**5.56**			E259G,**G1099S**

[a] Resistant MIC values are bolded and non-wild type isolates that have MIC values below the resistance breakpoints are in brackets.
[b] Results considered significant are bolded.
[c] Bolded alterations are GoF alterations described by Ferrari et al. [14].

in 2010 [8]. These numbers are much higher than the ones documented by the SENTRY Surveillance Program analysis [3], including this study, but our numbers highlight how resistance in this organism can increase.

The overexpression of ABC transport systems, including CgCDR1, CgCDR2, and CgSNQ2, have been described as the main fluconazole-resistance mechanism in *C. glabrata* clinical isolates [6]. In our study, we noted the overexpression of CgCDR1 and CgCDR2 individually or in combination in all but two isolates; however, we did not note the overexpression of CgSNQ2 as others reported [14,31]. A survey of 29 unmatched *C. glabrata* isolates from various infection sources collected in an Italian hospital also demonstrated that overexpression of CgCDR1 and CgCDR2 were the main mechanisms causing fluconazole resistance or fluconazole MIC values that were categorized as susceptible-dose dependent (SDD) [31]. In contrast to our study, the authors observed overexpression of CgSNQ2 in several isolates, including those with no overexpression of CgCDR1 and CgCDR2 [31]. Additionally, CgSNQ2 overexpression has been described among laboratory-generated *C. glabrata* strains that developed azole resistance due to voriconazole exposure [32]. Despite the findings of CgSNQ2 upregulated isolates, Whaley et al. demonstrated in a study designed to evaluate the importance of the upregulation of each ABC transporter that CgCDR1 is more important than CgCDR2 or CgSNQ2 for fluconazole resistance in *C. glabrata* [33].

The upregulation of the ABC transporters has been associated with the transcriptional regulator PDR1 [13]. GoF mutations in this transcriptional factor is responsible for high expression levels of CgCDR1, CgCDR2, or CgSNQ2, and the deletion of *PDR1* was demonstrated to restore azole susceptibility in *C. glabrata* [33]. PDR1 mutations are diverse and can be identified in different domains of this transcriptional factor. Notably, these GoF mutations not only regulate resistance pathways to azoles but also have been proven to increase fitness and virulence in *C. glabrata* [14]. The PDR1 GoF mutations described in eight isolates of this study were in the regions described as the putative inhibitory domain (amino acids 312 to 382) or the putative transcriptional activation domain (amino acids 800 to 1107) [13,14]. Additionally, two alterations were noted in the middle homology region (amino acids 539 to 632). According to Ferrari et al. [14], mutations in these domains confer azole resistance. Interestingly, the four remaining isolates that displayed amino acid alterations outside of these domains (amino acids 280 or 297) also exhibited elevated expressions of CgCDR1 and CgCDR2 that were 10-fold higher than the fluconazole-susceptible control.

Mutations in the genes encoding the target of azole agents, ERG11 encoding lanosterol 14α-demethylase, are not deemed prevelant in *C glabrata*, despite studies describing these mechanisms in clinical isolates. The clinical isolates in this collection did not harbour mutations in ERG11. Additionally, a recent study demonstrated that the transcriptional regulator CgRpn4 regulates the expression of 212 genes, including those that maintain the ergosterol level in the presence of fluconazole [34]. We did not investigate changes in this gene as part of this study.

Only three fluconazole-resistant *C. glabrata* isolates displayed resistance to the echinocandins, and one additional isolate had an intermediate MIC results to micafungin. All four echinocandin-nonsusceptible isolates displayed common alterations in the FKS HS sequences. Interestingly, none of the isolates resistant to fluconazole and nonsusceptible to the echinocandins, of which three were considered MDR, had alterations in the MSH2 hyper mutator factor that could potentially elevate the number of aleatory mutations and contribute to the emergence of MDR [35]. Despite the low prevalence of MDR isolates, due to the limited number of antifungal classes and options to treat infection caused by MDR *Candida* species, MDR rates should be closely monitored.

Azole resistance in *C. glabrata* is a product of selective pressure. The finding that a few STs were prevalent among fluconazole-resistant and geographically diverse *C. glabrata* isolates might suggest that certain STs could develop resistance more frequently. Furthermore, the analysis of fluconazole-resistant isolates from a 12-year survey in Belgium revealed that some isolates collected during a specific period were genetically identical, indicating clonal dissemination, but isolates collected in other points of the study were genetically distinct, suggesting the *de novo* acquisition of mutations [36]. The genetic profile of the fluconazole-resistant isolates collected from Spain in our study indicates similar events, with the emergence of resistance in different lineages as well as clonal dissemination.

In a 10-year study conducted in South Korean university hospitals to evaluate risk factors and mortality rates for fluconazole-resistant *C. glabrata* bloodstream infections [37], the authors concluded that the prior use of azoles was the most important. This conclusion was also seen in another investigation by Garnacho-Montero that analysed *C. glabrata* and other species causing candidemia [38].

Due to the prolonged antifungal regimens often recommended for prophylaxis or treatment of patients with invasive *Candida* infections [39], *C. glabrata* isolates might be exposed to an antifungal agent for extended periods of time. In this case, this haploid species might select for resistant organisms more frequently than other species that require two mutated alleles to develop resistance. Monitoring *C. glabrata* isolates for resistance to broadly used agents, such as those in the azole and echinocandin classes, and understanding the epidemiology and resistance mechanisms these isolates generate would provide knowledge to improve patient care, prevent dissemination of clonal isolates, and help development future antifungal agents active against MDR isolates.

Declaration of Competing Interests

JMI Laboratories was contracted to perform services in 2018–2021 for Achaogen, Inc., Affinity Biosensors, Albany College of Pharmacy and Health Sciences, Allecra Therapeutics, Allergan, Amicrobe Advanced Biomaterials, Inc., American Proficiency Institute, AmpliPhi Biosciences Corp., Amplyx Pharma, Antabio, Arietis Corp., Arixa Pharmaceuticals, Inc., Artugen Therapeutics USA, Inc., Astellas Pharma Inc., Athelas, Becton, Basilea Pharmaceutica Ltd., Bayer AG, Becton, Beth Israel Deaconess Medical Center, BIDMC, bioMerieux, Inc., bioMerieux SA, BioVersys Ag, Boston Pharmaceuticals, Bugworks Research Inc., CEM-102 Pharmaceuticals, Cepheid, Cidara Therapeutics, Inc., Cipla, Contrafect, Cormedix, Inc., Crestone, Inc., Curza, CXC7, DePuy Synthes, Destiny Pharma, Dickinson and Company, Discuva Ltd., Dr. Falk Pharma GmbH, Emery Pharma, Entasis Therapeutics, Eurofarma Laboratorios SA, Fedora Pharmaceutical, F. Hoffmann-La Roche Ltd., Fimbrion Therapeutics, US Food and Drug Administration, Fox Chase Chemical Diversity Center, Inc., Gateway Pharmaceutical LLC, GenePOC Inc., Geom Therapeutics, Inc., GlaxoSmithKline plc, Guardian Therapeutics, Hardy Diagnostics, Harvard University, Helperby, HiMedia Laboratories, ICON plc, Idorsia Pharmaceuticals Ltd., IHMA, Iterum Therapeutics plc, Janssen Research & Development, Johnson & Johnson, Kaleido Biosciences, KBP Biosciences, Laboratory Specialists, Inc., Luminex, Matrivax, Mayo Clinic, Medpace, Meiji Seika Pharma Co., Ltd., Melinta Therapeutics, Inc., Menarini, Merck & Co., Inc., Meridian Bioscience Inc., Micromyx, Microchem Laboratory, MicuRx Pharmaceutics, Inc., Mutabilis Co., N8 Medical, Nabriva Therapeutics plc, National Institutes of Health, NAEJA-RGM, National University of Singapore, North Bristol NHS Trust, Novartis AG, Novome Biotechnologies, Oxoid Ltd., Paratek Pharmaceuticals, Inc., Pfizer, Inc., Pharmaceutical Product Development, LLC, Polyphor Ltd., Prokaryotics, Inc., QPEX Biopharma, Inc., Ra Pharmaceuticals, Inc., Rhode Island Hospital,

RIHML, Roche, Roivant Sciences, Ltd., Safeguard Biosystems, Salvat, Scynexis, Inc., SeLux Diagnostics, Inc., Shionogi and Co., Ltd., SinSa Labs, Specific Diagnostics, Spero Therapeutics, Summit Pharmaceuticals International Corp., SuperTrans Medical LT, Synlogic, T2 Biosystems, Taisho Pharmaceutical Co., Ltd., TenNor Therapeutics Ltd., Tetraphase Pharmaceuticals, The Medicines Company, The University of Queensland, Theravance Biopharma, Thermo Fisher Scientific, Tufts Medical Center, Universite de Sherbrooke, University of Colorado, University of Southern California-San Diego, University of Iowa, University of Iowa Hospitals and Clinics, University of North Texas Health Science Center, University of Wisconsin, UNT System College of Pharmacy, URMC, UT Southwestern, VenatoRx, Viosera Therapeutics, Vyome Therapeutics, Inc., Wayne State University, Wockhardt, Yukon Pharmaceuticals, Inc., Zai Lab, and Zavante Therapeutics, Inc. There are no speakers' bureaus or stock options to declare.

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Ethical approval

Not required.

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