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Azole Resistance in *Candida glabrata* Clinical Isolates from Global Surveillance is Associated with Efflux Overexpression

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Introduction

- *Candida glabrata* is the second most common *Candida* species causing human infections, only after *Candida albicans*.
- Azole resistance among *C. glabrata* has been reported to be as high as 10%.
- The SENTRY Antifungal Surveillance Program documented an increase of 8.6% to 10.1% in fluconazole-resistant *C. glabrata* isolates from 1996 to 2014.
- Azole resistance can arise during therapy due to induction of overexpression of efflux pumps.
- Alterations in ERG11, the target of azole agents, have also been described in *C. glabrata*.
- In this study, we evaluated azole resistance mechanisms and epidemiology of fluconazole-resistant *C. glabrata* isolates in the SENTRY Program during 2018 and 2019.

Materials and Methods

- A total of 2,992 invasive fungal, consecutive, and non-duplicated clinical isolates were collected during 2018 and 2019 as part of a global surveillance initiative in 68 hospitals located in 28 countries.
  - Isolates were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry and molecular methods.
- Susceptibility testing was performed for using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution reference method.
  - CLSI M60 (2017) clinical breakpoints and CLSI M59 (2016) epidemiologic cutoff values (ECVs) were applied.
- Quality control was performed as recommended in CLSI M60 (2017) guidelines.
  - All results were within established ranges.
- *C. glabrata* isolates displaying resistance to fluconazole were submitted to whole genome sequencing on a MiSeq Sequencer (Illumina, San Diego, CA, USA).
  - *ERG11*, *MDR1*, and *CDR1* sequences were analysed and compared to those of *C. glabrata* ATCC 60032 and *C. glabrata* ATCC 93330.
- The expression of *ERG11*, *CDR1*, and *MDR1* was determined by quantitative real-time PCR (qRT-PCR) using high quality DNA-free mRNA preparations.
  - Relative quantification of target genes was performed in triplicate by normalization to an endogenous reference gene (18S).
  - Transcription levels were considered significantly different if a 10-fold difference was noted compared to *C. glabrata* ATCC MYA2950.

Results

- Fluconazole resistance was noted among 34 (6.1%) of the 561 *C. glabrata* isolates collected worldwide.
  - Fluconazole resistance varied geographically from 0% in Latin America to 2.7% in Asia-Pacific, 5.9% in Europe, and 8.1% in North America (Figure 1).
- Sixteen (16/34; 47.1%) of the fluconazole-resistant *C. glabrata* isolates were recovered in US hospitals.
- All fluconazole-resistant *C. glabrata* isolates were non-wild type for voriconazole, 24/34 were non-wild type to posaconazole, but only 2 were non-wild type to itraconazole.
  - Isavuconazole MIC values ranged from 0.25 to >4 mg/L.
- Fourteen different sequence types (ST) were noted among fluconazole-resistant *C. glabrata* isolates (Table 1).
  - The most common ST was ST3 (n = 7), which was observed in 2 countries: Spain and the US.
  - ST7 or the single locus variant ST200 (n = 5) was noted in 5 countries.
  - ST6 isolates (n = 4) was noted in 2 countries: Slovenia and Spain.
- All isolates displayed wildtype sequences for ERG11 and ERG3.
- CDR1 overexpressed 10X to 85X among 20 fluconazole-resistant *C. glabrata* isolates (Table 1).
  - Two isolates displayed a modest increase in the CDR1 expression of 5X and 8X the control.
- Eighteen isolates overexpressed CDR2 (>10X).
- Eight isolates had CDR2 expression rates ranging from 5X to 9X the control.
- Fourteen isolates showed an increase in the expression of CDR1 and CDR2.
- Fourteen isolates showed Gain of function (GoF) alterations in transcription factor PDR1 (Table 1).
- All isolates displayed a basal expression of SNQ2 and ERG11.
- Only 1 isolate from Korea did not have a resistance mechanism identified.
- Ten isolates belonging to STs 2, 7, 8, 11, and 200 had a MSH2 mutator genotype (V239L).
- Four (11.8%) isolates that were non-susceptible to one or more echinocandins that had a FKS2 HS1 alteration (2 S663P and 2 F659Y/del) (Table 1).

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

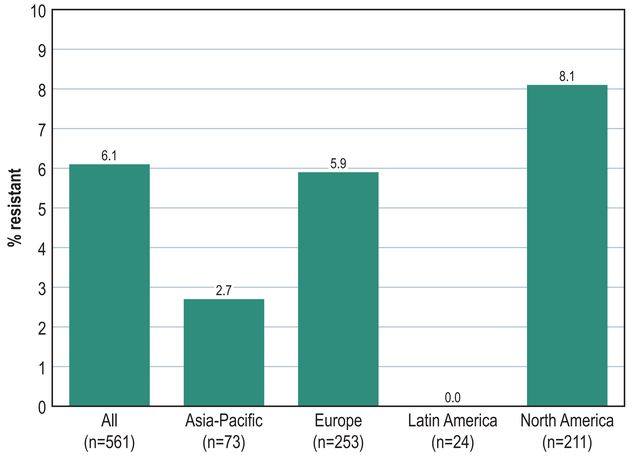

Table 1 Susceptibility profiles and genetic characteristics of fluconazole-resistant *C. glabrata*

Country	ST	Study Year	MIC (mg/L)								Gene expression relative to control		Sequence alterations [a]		
			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
UK	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_del		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	8	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] Alterations in bold are GoF alterations described by Ferrari et al. (2009)

Conclusions

- Fluconazole resistance among *C. glabrata* was higher in North America than other regions.
  - These isolates were not observed in Latin America.
- Overexpression of CDR1 and/or CDR2 was noted in 33 of 34 isolates evaluated.
- PDR1 alterations associated to GoF were noted in 14 isolates.
- As azole resistance in *C. glabrata* is a product of selective pressure, the finding that just a few STs were prevalent among geographically-diverse fluconazole-resistant *C. glabrata* isolates suggests that certain STs could develop resistance more frequently.

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