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Azole Resistance in *Candida parapsilosis* and *Candida tropicalis* from a Global Surveillance Is Mainly Caused by Alterations in Erg11 and MDR1 Overexpression

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Introduction

- Globally, non-albicans *Candida* species have been increasingly reported and their susceptibility profiles have shown remarkable geographic variations
- High rates of fluconazole-resistant *C. glabrata* have been reported from sentinel and population-based surveillance
- Fluconazole resistance is generally considered to be uncommon among *C. parapsilosis* and *C. tropicalis* isolates, but fluconazole-resistant *C. parapsilosis* and *C. tropicalis* isolates are reported in certain geographic areas
  - These isolates may emerge following drug pressure in the form of fluconazole treatment and prophylaxis with subsequent patient-to-patient transmission within the hospital environment
- Azole resistance in *Candida* species is usually caused by the increased expression of the target of these agents involved in the ergosterol synthesis pathway, overwhelming the number of antifungal molecules present
  - The action of efflux systems that remove the antifungal agent from the intracellular space is also an important azole resistance mechanism in *Candida* species
- We evaluated the activity of fluconazole and voriconazole against a global collection of *Candida* spp. isolates and characterized azole resistance mechanisms in *C. parapsilosis* and *C. tropicalis*

Materials and Methods

- A total of 2,825 invasive fungal, consecutive, and non-duplicated clinical isolates were collected as part of a global surveillance initiative in 59 hospitals located in 25 countries
  - Isolates were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry and molecular methods as described elsewhere
- Susceptibility testing was performed for fluconazole, posaconazole, and voriconazole using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution reference method
- CLSI clinical breakpoints were used for the most common species of *Candida* (M60 guidelines), and CLSI M59 recently published epidemiologic cutoff values (ECVs) were applied for species without breakpoints
- Quality control was performed as recommended in CLSI M60 guidelines and all results were within established ranges
- *C. parapsilosis* and *C. tropicalis* isolates displaying resistance to fluconazole and/or voriconazole 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. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 750
- 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 with *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 750

Results

- Resistance to fluconazole and voriconazole was detected among 0.4% and 0.1% of the *C. albicans*
  - Azole-resistant *C. albicans* were not observed in Asia-Pacific countries and were more frequent in North America (1.1%) and Latin America (1.0%) when compared to Europe (0.1%)
  - A total of 3.1% of the isolates were non-wild type to posaconazole
- Fluconazole resistance was noted among 6.5% of *C. glabrata* isolates, but the rate ranged from a high of 13.0% in North America to 0.0% in Latin America
  - Applying ECVs, 89.4% and 95.1% of *C. glabrata* isolates were wild type to voriconazole and posaconazole, respectively (Figure 1)
- Fluconazole and voriconazole susceptibility rates for *C. parapsilosis* isolates were 89.1% and 91.6%, respectively
  - Azole resistance among *C. parapsilosis* was highest among isolates from Europe (15.0%) and was not observed in Asia-Pacific or Latin America
- Fluconazole resistance in *C. tropicalis* isolates was more common in Asia-Pacific (8.3%), when compared to Latin America, (3.2%), Europe (1.8%), and North America (0.0%)
- Voriconazole was active against 94.7% of *C. krusei* isolates; resistance was only observed in North America (5.0%)
- Fluconazole non-wild-type isolates were noted among 3/77 (3.9%) *C. dubliniensis*, 4/17 (23.5%) *C. guilliermondii*, and 4/47 (8.5%) *C. lusitaniae*
- *C. auris* (1 isolate), *C. blankii* (1), *C. duobushaemulonii* (1), *C. inconspicua* (1), *C. norvegensis* (1), 3/12 of *C. fermentati* isolates, and 4/8 of *C. pelliculosa* isolates displayed elevated fluconazole MIC values at ≥16 mg/L and voriconazole MIC values at ≥0.5 mg/L (Figure 2)
- Most of the 46 *C. parapsilosis* isolates displaying nonsusceptible or non-wild-type fluconazole or voriconazole MIC values, were from Italy (32/46) and had the Y132F alteration on Erg11 and overexpressed the MDR1 (Table 1)
- Among 11 *C. parapsilosis* isolates from other countries, 2 isolates had Y132F Erg11 alteration alone and 3 isolates had Y132F Erg11 alteration with overexpression of MDR1 or CDR1
  - Overexpression of Erg11, Erg11 alteration or MDR1 overexpression alone or with CDR1 were noted in the remaining 5 isolates
  - One isolate from France had no resistance mechanism identified
- Three *C. tropicalis* isolates from Thailand were also resistant to voriconazole and harboured the same Erg11 alterations Y132F and S154F
- The 4 isolates from other countries had lower fluconazole MIC results (4–16 mg/L) when compared to the isolates from Thailand (32–64 mg/L) and did not display any of the tested azole resistance mechanisms

Conclusions

- Geographic differences in azole resistance among the most common *Candida* species are important to guide therapy and indicate the need to adjust empirical antifungal coverage when resistance is known to occur in that region or institution
- Azole resistance in *C. parapsilosis* and *C. tropicalis* was mainly caused by Erg11 mutations in position 132
  - This alteration has also been reported among other *Candida* species and could be evaluated as a target for a rapid test to detect resistance in these species
- Azoles are still active against the most common *Candida* spp. and resistance seems to be restricted to certain organisms in specific geographic regions, mainly *C. glabrata* in the United States and *C. parapsilosis* in Italy

Figure 1 Azole susceptibility profiles of the most common yeast species (>10 isolates)

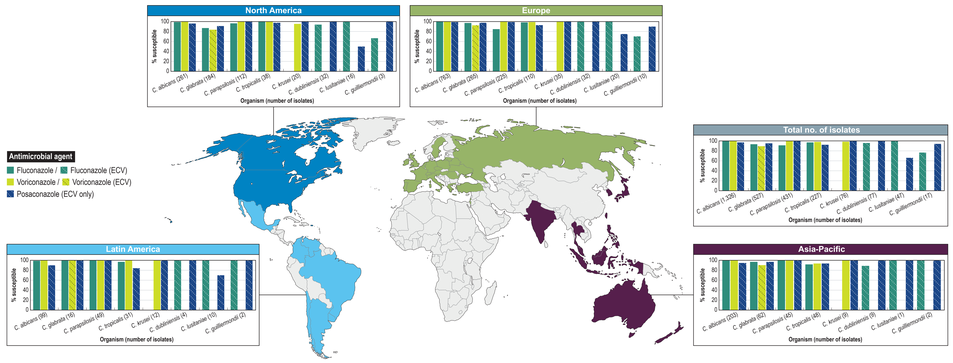
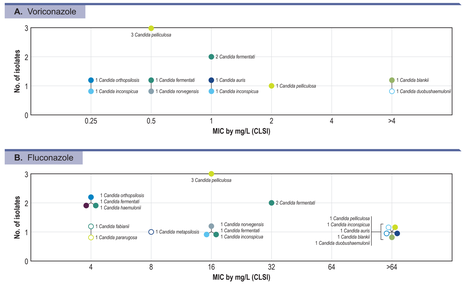


Table 1 Characterization of *C. parapsilosis* and *C. tropicalis* azole resistance mechanisms

Year	Country, state or city	MIC (mg/L)		Amino acid substitutions			Relative quantification			Genotype
		Fluconazole	Voriconazole	Erg11	Mdr1	CDR1	CDR1	Erg11	MDR1	
Candida parapsilosis										
27 isolates										
2016-2017	Italy	32-64	0.25-0.5	Y132F			0.79-3.57	1.64-5.2	36.3-125.96	*erg11* mutation, MDR1 overexpression
Single isolates										
2016	Italy	2	0.25	Y132F			2.27	0.98	34.29	*erg11* mutation, MDR1 overexpression
2016	Italy	32	0.5	Y132F			2.07	13.59	380.63	*erg11* mutation, Erg11 overexpression, MDR1 overexpression
2016	Italy	128	1	Y132F, R398I	I396V	S79N	0.94	2.20	49.46	*erg11* mutation, MDR1 overexpression
2017	Italy	4	0.06	R398I	I396V		1.59	1.69	2.12	none
2017	Italy	4	0.06				2.04	1.44	4.79	none
2017	Italy	8	0.12	Y132F			2.19	1.54	52.69	*erg11* mutation, MDR1 overexpression
2017	Italy	8	0.25	Y132F			2.53	1.54	60.58	*erg11* mutation, MDR1 overexpression
2017	Italy	16	0.25	Y132F			0.77	1.68	57.12	*erg11* mutation, MDR1 overexpression
2016	Czech Republic	4	0.06	R398I	I396V		4.96	0.38	14.69	MDR1 overexpression
2017	France	4	0.12				3.14	2.93	0.13	none
2017	France	32	0.5	Y132F			2.08	1.76	42.52	*erg11* mutation, MDR1 overexpression
2016	Ireland	8	0.12	R398I	I396V		2.04	10.34	6.59	Erg11 overexpression
2016	South Korea	4	0.06	Y132F	V175M	G151S, V153L, N163D	1.40	1.09	20.76	*erg11* mutation, MDR1 overexpression
2016	USA, California	4	0.12				22.51	0.94	18.15	CDR1 overexpression, MDR1 overexpression
2017	USA, California	32	0.5	Y132F			4.38	2.34	2.92	erg11 mutation
2017	USA, Indiana	32	0.5	Y132F			18.90	0.33	3.75	*erg11* mutation, CDR1 overexpression
2016	USA, New York	8	0.12				1.68	1.72	43.67	MDR1 overexpression
2017	USA, New York	4	0.06	K143R			4.57	1.92	3.03	*erg11* mutation
2017	USA, Texas	8	0.25	Y132F			6.22	3.40	6.28	*erg11* mutation
Candida tropicalis										
2016	Czech Republic	16	1		E133D		0.83	1.58	5.31	none
2016	France	8	0.25		V76A		0.89	0.32	0.86	none
2016	Mexico	16	0.25				2.80	1.40	2.35	none
2016	New Zealand	4	0.25		A189V		1.53	1.04	1.81	none
2016	Thailand	32	1	Y132F, S154F		0.88	2.31	2.10		*erg11* mutation
2016	Thailand	64	4	Y132F, S154F		1.54	4.87	1.89		*erg11* mutation
2017	Thailand	32	1	Y132F, S154F		1.06	3.06	1.77		*erg11* mutation

Significant changes in expression are underlined.

Figure 2 Uncommon *Candida* species displaying voriconazole MIC values ranging from 0.25 to >4 mg/L or fluconazole MIC values ranging from 4 to >64 mg/L



Acknowledgements

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