Antifungal susceptibility of *Aspergillus sp*., *Scedosporium sp*. and *Exophiala sp.* isolated from patients with cystic fibrosis

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Purpose

Molds are frequently recovered from respiratory samples of cystic fibrosis (CF) patients. During the last decade, antifungal resistance in non-*Candida* species is increasingly reported. The emergence of azole resistance in *Aspergillus fumigatus* is an increasing problem. Azole resistance in filamentous fungi is mostly mediated by point mutations in the *cyp51A* gene, which encodes lanosterol 14α-demethylase, the target of azole antifungals. The aim of this work is to overview the susceptibility profile from different fungal species isolated from respiratory secretions of Argentinean CF patients and to analyze the underlying molecular mechanisms of azole resistance in the isolated *Aspergillus fumigatus* strains.

Methods and Materials

Amphotericin B (AMB), itraconazole (ITC), posaconazole (PSC) and voriconazole (VCZ) minimum inhibitory concentrations (MICs) for 70 fungal strains (53 *Aspergillus sp*., 12 *Scedosporium sp*. and 5 *Exophiala sp*.) recovered from 32 CF patients were determined by using the CLSI M38-A2 broth dilution method. The mechanism of azole resistance in *A. fumigatus*, was investigated by PCR amplification and sequence analysis of the full *Cyp51A* coding sequences and its 5´UTR region.

Results

For AMB, MICs were above the epidemiological cut-off values (ECV) in 26%, 50% and 60% of the *Aspergillus sp*., *Scedosporium sp*. and *Exophiala sp*., respectively. For VCZ, MICs were above the ECV in 10% and 42% of *Aspergillus* and *Scedosporium* species, respectively. For PSC, MICs were above the ECV in 8%, 17% and 20% of *Aspergillus*, *Scedosporium* and *Exophiala* species, respectively. For ITC, MICs were above the ECV in 8% and 83% of *Aspergillus* and *Scedosporium* species, respectively (Table 1). Those *A. fumigatus* strains with MICs values higher than their corresponding ECVs for azole drugs (Table 2) were checked for mutations in the *Cyp51A* gene. Results showed that the three isolates with high azole MIC values harbor 34-bp tandem repeat of 34 (TR34) (Figure 1) in the promoter region, a L98H mutation at codon 98 (TR34/L98H) and a S297T amino acid substitution (Figure 2). Moreover, Isolate # 37 showed the described Cyp51Ap substitutions together with a R65K mutation. This last strain showed a MIC value for VCZ higher than ECV (Table 3).

Isolates (n)	n=	Frecuencia de CIM (ug/ul)									
A. flavus (7)	**7**	**0.015**	**0.03**	**0.06**	**0.1**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
	AMB	0	0	0	0	0	1	2	0	0	4
	VCZ	0	0	0	0	0	6	1	0	0	0
	PSC	2	1	0	0	2	2	0	0	0	0
	ITC	3	0	0	0	1	3	0	0	0	0
A. fumigatus (27)	**27**	**0.015**	**0.03**	**0.06**	**0.1**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
	AMB	0	0	0	0	0	15	6	1	1	4
	VCZ	0	0	0	0	10	14	2	1	0	0
	PSC	7	1	4	3	3	6	0	2	1	0
	ITC	2	4	7	1	1	7	2	0	0	3
A. niger (5)	**5**	**0.015**	**0.03**	**0.06**	**0.1**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
	AMB	0	0	0	0	0	2	2	0	1	0
	VCZ	0	0	0	0	2	3	0	0	0	0
	PSC	0	0	1	0	1	3	0	0	0	0
	ITC	0	0	1	0	0	1	1	2	0	0
A. terreus (10)	**10**	**0.015**	**0.03**	**0.06**	**0.1**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
	AMB	0	0	0	0	0	3	2	1	1	2
	VCZ	0	0	0	1	1	1	3	3	0	0
	PSC	5	0	0	2	2	0	0	0	0	0
	ITC	5	2	0	1	0	1	0	0	0	0
Exophiala sp. (5)	**5**	**0.015**	**0.03**	**0.06**	**0.1**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
	AMB	0	0	0	0	1	0	1	1	2	0
	VCZ	0	0	0	0	1	1	2	1	0	0
	PSC	0	0	0	2	0	1	1	1	0	0
	ITC	0	1	1	2	0	0	1	0	0	0
Scedosporium spp. (12)	**12**	**0.015**	**0.03**	**0.06**	**0.1**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
	AMB	0	0	0	0	0	1	1	1	2	6
	VCZ	0	0	0	0	1	2	3	3	2	0
	PSC	0	0	0	0	1	0	1	3	4	2
	ITC	1	0	0	0	0	0	0	0	0	10
A. calidoustus (2)	**2**	**0.015**	**0.03**	**0.06**	**0.1**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
	AMB	0	0	0	0	0	0	0	1	1	0
	VCZ	0	0	0	0	0	0	0	0	0	2
	PSC	0	0	0	0	0	0	0	0	0	2
	ITC	0	0	0	0	0	0	0	0	0	2
A. lentulus (1)	**1**	**0.015**	**0.03**	**0.06**	**0.1**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
	AMB	0	0	0	0	0	0	0	0	0	1
	VCZ	0	0	0	0	0	0	0	0	0	1
	PSC	0	0	0	0	0	0	1	0	0	0
	ITC	0	0	0	0	0	0	0	0	0	1
A. parasiticus (1)	**1**	**0.015**	**0.03**	**0.06**	**0.1**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
	AMB	0	0	0	0	0	0	0	0	0	1
	VCZ	0	0	0	0	0	1	0	0	0	0
	PSC	0	0	0	0	1	0	0	0	0	0
	ITC	0	0	0	0	1	0	0	0	0	0

Table 2 – Frequency of CIM distribution of isolates. Cells in red shading correspond to values above the ECV.

Percentage of *Aspergillus* isolates above the ECV				
Total = 70	AMB	VCZ	PSC	ITC
A. fumigatus	5	1	3	3
A. flavus	4	0	0	0
A. niger	1	0	0	0
A. terreus	2	3	0	0
A. lentulus	1	1	1	1
Sub-Total	13	5	4	4
%	26%	10%	8%	8%

Table 1 – Percentage of *Aspergillus* isolates with MICs above ECV.

Isolate #	**MICs (µg/µl)**				***CYP51A* Promotor**	**Cyp51Ap aa. Substitutions at residue**		
	AMB	**VCZ**	**PSC**	**ITC**		**R65**	**L98**	**S297**
5	0.50	1.00	2.00	8.00	TR34	WT	L98H	S297T
37	0.50	2.00	4.00	8.00	TR34	R65K	L98H	S297T
60	0.50	1.00	2.00	8.00	TR34	WT	L98H	S297T

Table 3 – MICs values and mutation profiles for the three non-WT *A. fumigatus*. Red shaded MIC values are higher than the described ECVs. AMB: amphotericin B, VCZ: voriconazole, PSC: posaconazole, ITC: itraconazole. WT: wild type.

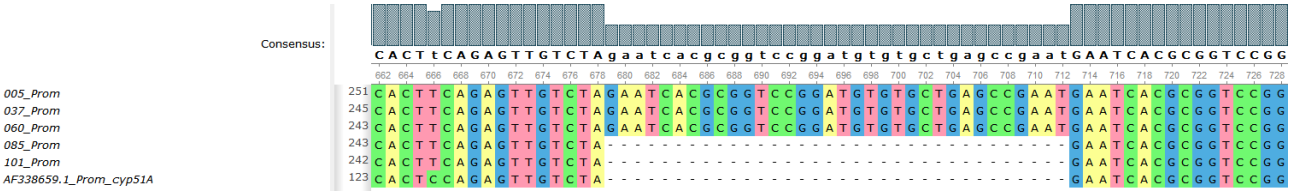

Figure 1 – Alignment of promoter region of the three non-WT *A. fumigatus* with reference sequence showing the presence of the TR34.

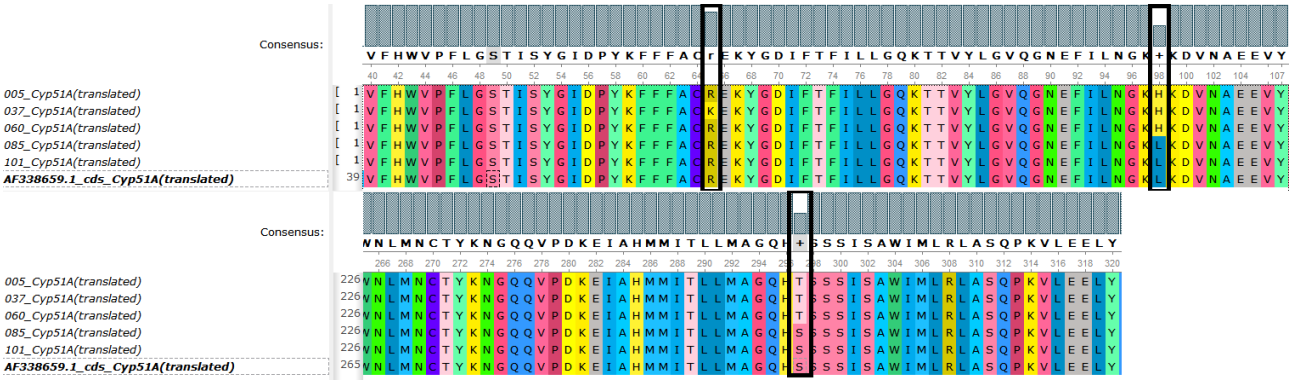

Figure 2 – Alignment of the translated coding sequence of*Cyp51A*of the three non-WT *A. fumigatus* with reference sequence (AF338659.1). Point mutations can be observed in amino acid positions65, 98 and 297 with respect to reference sequence.

Conclusion

This is one of the few susceptibility profile studies for antifungal drugs in molds from CF patients in Argentina. We found that some *Aspergillus* spp. isolates have a reduced susceptibility to ITC (8%), PSC (8%) and VCZ (10%). Also, we found that 26% of the strains showed high AMB MIC values. Similarly, Scedosporium spp. showed high MIC values for all tested drugs in 50%, 42%, 17%, 83% of the strains for AMB, VCZ, PSC and ITC, respectively. In contrast, azoles showed good in vitro activity against *Exophiala* spp. All ITC/PSC-non-wild type *A. fumigatus* isolates showed one of the most frequently described resistance mechanism in *A. fumigatus* worldwide (TR34/L98H). Since theTR34/L98H mutant strains are reported to be of environmental origin and to be a consequence of the use of fungicides in agriculture, further studies are needed to confirm this environmental hypothesis in our country. The R65K amino acid substitution in Cyp51Ap role in azole resistance phenotype should be studied. Finally, azole-resistance is an emerging problem that should be continuously monitored in CF patients.

References

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