Molecular genetic analysis of the 14-alpha lanosterol demethylase gene in azole-resistant pathogenic fungi

P 1620

Taraskina A.E., Dmitrriev K.A., Vybornova I.V., Raush E.R., Latypova E.M., Pchelin I.M., Vasileva N.V.
Kashkin Research Institute of Medical Mycology, North-Western State Medical University named after I.I. Mechnikov, Saint-Petersburg, Russia
ataraskina@mail.ru

Background

Fungal pathogens cause life-threatening infections in immunocompetent and immunocompromised individuals. The most prevalent fungal pathogens are *Candida* spp. and *Aspergillus* spp. Azole drugs are the fist line of therapy against them. Antifungal drug resistance is a confounding factor that negatively impacts clinical outcome for patients with serious mycoses. Recent years have seen the growth of molecular technology that is ideally suited for assessment of drug resistance mechanisms. The target of the azoles is the 14-alpha lanosterol demethylase enzyme generated by the gene *erg11* in yeasts and the *cyp51* in filamentous fungi [1]. Reduction of the susceptibility to azole is mainly triggered by point mutation in this gene. Nowadays, multiple point mutations in *cyp51*/*erg11* are identified. However, not all of them are accounted for the drug-resistant phenotype and they are geographically different [2, 3].

The aim of the study is a molecular genetic analysis of the *cyp51*/*erg11* nucleotide sequence for azole-resistant strains *Candida* spp. and *Aspergillus* spp. isolated on the territory of the Russian Federation, which can serve as a basis of molecular diagnostic platforms suitable for rapid detection of drug resistance.

Referens

1. Xie J.L., Polvi E.J., Shekhar-Guturja T., Cowen L.E. Elucidating drug resistance in human fungal pathogens. Future Microbiol. (2014). 9(4): 523-542.
2. Vermeulen E, Lagrou K, Verweij PE. Azole resistance in *Aspergillus fumigatus*: a growing public health concern. Curr Opin Infect Dis (2013). 26: 493-500.
3. Ford C.B., Funt J.M., Abbey D. et al. The evolution of drug resistance in clinical isolates of *Candida albicans*.eLife 2015;4:e00662.

Materials and methods

All *Candida* and *Aspergillus* isolates were identified by sequencing of internal transcribed spacer region (ITS) and fraction of the β-tubulin gene. Antifungal susceptibility testing was carried out according to the EUCAST microdilution method (table 1).

Table 1. Clinical *Candida* spp. and *Aspergillus* spp. isolates

Clinical isolate	Molecular identification	Resistance to
RCPF-1592	C. *albicans*	FLU
RCPF-1274	C. *albicans*	FLU, VRC, ITR, KTZ
Ch №1	A.*terreus*	VRC
RCPF-111/67	A.*terreus*	S
Kr №2	A.*flavus*	VRC, ITR
Pav №3	A.*flavus*	KTZ
RCPF-1382/800	A.*flavus*	VRC
RCPF-1247/1094	A.*flavus*	S
Bar №4	A.*niger*	KTZ
RCPF-1249/800-2	A.*niger*	S
RCPF-1345	A.*niger*	nd
Kor №5	A.*calidoustus*	VRC, ITR
RCPF-11/266	A.*calidoustus*	nd

FLU – fluconazole; ITC – itraconazole; VRC – voriconazole; KTZ – ketoconazole; S – susceptibility; ND – not determined

The nucleotide sequences of pathogenic *Candida* spp. and *Aspergillus* spp. *cyp51*/*erg11* were aligned with reference sequence from GenBank database (http://www.ncbi.nlm.nih.gov/genbank/).

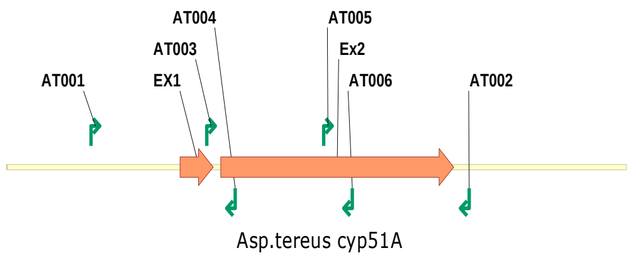

Fig 1. Example of sequencing design

Optimal oligonucleotides sequences were selected for amplification and sequencing of *Candida* spp. and *Aspergillus* spp. *cyp51*/*erg11*. Specificity of the oligonucleotides was verified by BLAST algoritm (http://www.ncbi.nlm.nih.gov/blast/)

Results

We currently included in the study 12 and 2 resistant clinical *Aspergillus* spp. and *Candida* spp isolates, respectively, which belong to the following species: *A.terreus*, *A.flavus*, *A.niger, A. calidoustus* and *C.albicans* (table 1).
The erg11 gene of the resistant strain *Candida albicans* compared with the standard sequence of wild type gene X13296.

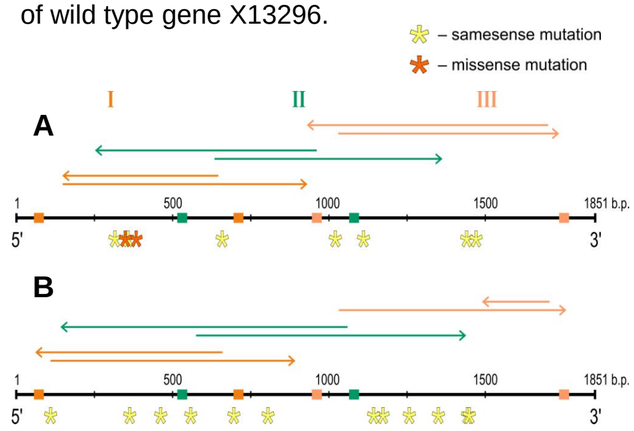

Fig 2. Sequencing design and nucleotide substitutions in the erg11 gene from *C.albicans* clinical isolates (A – RCPF1274; B – RCPF1592)

The *erg11* gene of the strain *C. albicans* **RCPF-1274** bears the following seymens mutations:T315C, A357G, C658T, A1020G, C1110T, A1440G, A1470C and missense mutation T348A and A383C lead to substitutions D116E, K128T (fig.2 A).
The *erg11* gene of the strain *C. albicans* **RCPF-1592** bears the following samesense mutations: T110Y, C363Y, T462C, C558T, T696Y, C805T, T1143C, A1173G, C1257T, T1350C, C1443T, T1449C (fig. 2 B).

The nucleotide sequence analysis of the *cyp51A* gene of *Aspergillus* isolates showed some point mutations in azole-resistant atrains (table 2). A resistant clinical isolate A.terreus had a most nucleotide polymorphism.

Table 2. Amino acide sequence analysis of CYP51A clinical *Aspergillus* spp. isolates

	Clinical isolate	Amino acide substitutions			
A. flavus	EQ963473	-			
	RCPF-1247/1094	-			
	RCPF-1382/800	-			
	Kr №2	A205T			
	Pav №3	A205T			
A. terreus	ATEG_05917.1	-	-	-	-
	RCPF-111/67	-	-	-	-
	Ch №1	S8T	L26F	K64R	Q277R
	ATEG_05917.1	-	-	-	
	RCPF-111/67	-	-	-	
	Ch №1	V336A	V390M	Q419H	
A. niger	An11g02230	-	-	-	-
	RCPF-1249/800-2	-	Q228R	E380Q	H506N
	RCPF-1345	T57A	Q228R	-	H506N
	Bar №4	T57A	Q228R	-	H506N

Conclusion

The study of the mutations in *cyp51*/*erg11* will help to determine molecular genetic characteristics of azole resistant *Aspergillus* and *Candida* isolates that may lead to development of original system for testing antimycotics resistant in human fungal pathogen.

26th ECCMID Amsterdam, Netherlands 9 – 12 April 2016
The congress of ESCMID