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Abstract (oral session)

Unique multidrug-resistant Candida albicans developed in vivo associated with well-known mutations in FKS1 and ERG11

R.H. Jensen*, M.C. Arendrup (Copenhagen, DK)

Objectives: Long term antifungal treatment implies a risk for selecting resistant fungi in vivo. Here we report the step-wise selection of a multi-drug class resistant C. albicans infection in a patient with oro-pharyngeal and oesophageal candidiasis over a 5-year period. Methods: Eight consecutive clinical isolates were received for routine culture and susceptibility testing. Susceptibility testing was done according to EUCAST E.def 7.2 (azoles, mica- and anidulafungin) and by Etest (amphotericin and caspofungin). Four isolates were stored and available for molecular analysis (Table). Two control strains (ATCC 64550 and 10231) were included. The FKS1 and ERG11 genes were sequenced and compared to reference sequences (GenBank; FKS1: XM_716336 and ERG11: X13296). Multi-locus sequence genotyping (MLST) was performed with 7 genes: AAT1a, ACC1, ADP1, MP1b, SYA1, VPS13 and ZWF1b and analysed with the pubMLST database (http://calbicans.mlst.net/). Results: The MICs are shown in Table. Fluconazole (FLC) resistance (R) was detected in isolate #3, 21 months after the first isolate was obtained. Itra-, posa- and voriconazole resistance was first detected in isolate #5 (month 48), echinocandin resistance in isolate 6 and amphotericin resistance in isolate 7 and 8 (month 54 and 55). Four mutations were found in the ERG11 gene of the FLC R isolate (Table). An additional ERG11 mutation (A61E) was found in the pan-azole resistant isolates. Isolate #4 and #5 also had a heterozygous mutation in FKS1 (V661V/F) but no increase in echinocandin MIC’s. The multidrug resistant isolates #7 and #8 both carried a heterozygous FKS1 mutation (S645S/P). MLST analysis indicated genetic relatedness among the clinical isolates, as 5 out of the 7 diploid gene sequences were 100 % identical. Three base pair differences on just one allele were observed in the SYA1 gene for isolate #5, while 10 differences also on one allele were found in MP1b for isolate #4 and #5. Both control strains were genetically distinct. Conclusion: To our knowledge, this is the first report dissecting the development of multidrug resistance in C. albicans. Azole and echinocandin resistance was linked to hot spot mutations in the FKS1 and ERG11 target genes, respectively. The mechanism responsible for the amphotericin resistance is currently being investigated. The findings clearly illustrate the challenge we face due to the limited antifungal armamentarium particularly for patients with long term fungal infections.

Table: Overview of the eight clinical *C. albicans* isolates and reference strains including susceptibility and molecular data.

Sample	Specimen	Genotype[$]	Sequence profiles of resistance genes			EUCAST (EDef 7.2) (µg/ml)					Etest (µg/ml)	
Collected	Origin	Allelic profiles	*FKS1*-HS1	*FKS1*-HS2	*ERG11*	POS	ANI	VOR	ITR	FLU	AMB	CAS
#1: 25.04.06	Oesophagus biopsy	NA	NA	NA	NA	NA	NA	≤ 0.03	≤ 0.03	0.125	0.25	0.06
#2: 11.07.06	Oesophagus biopsy	NA	NA	NA	NA	NA	NA	≤ 0.03	≤ 0.03	0.25	0.5	0.25
#3: 28.01.08	Pharynx swab	NA	NA	NA	NA	0.03/4*	NA	0.03/4*	0.03/4*	16	0.38	0.25
#4: 01.04.08	Mouth swab	[**21,26,14**,18,72,**65,84**]	V661V/F	WT	E266D, G307S, G450E, V488I	≤ 0.03	0.015	≤ 0.03	≤ 0.125	8	0.5	0.25
#5: 21.04.10	Oesophagus biopsy	[**21,26,14**,18,76,**65,84**]	V661V/F	WT	**A61E**, E266D, G307S, G450E, V488I	> 4	0.015	1	16	> 16	0.5	0.50
#6: 17.08.10	Oesophagus biopsy	NA	NA	NA	NA	NA	0.25	0.5	NA	> 16	0.5	> 32
#7: 10.04.11	Faeces	[**21,26,14**,19,72,**65,84**]	**S645S/P** V661V/F	WT	**A61E**, E266D, G307S, G450E, V488I	4	1	0.125	16	> 16	> 32	> 32
#8: 06.05.11	Colon biopsy	[**21,26,14**,19,72,**65,84**]	**S645S/P** V661V/F	WT	**A61E**, E266D, G307S, G450E, V488I	0.5**	0.5	0.125	> 16	16	> 32	> 32
ATCC 64550	ATCC	[2,2,5,2,2,25,5]	WT	WT	D116E, K128T, Q474K	0.5	0.015	0.5	1	16	0.50	0.25
ATCC 10231	ATCC	[35,7,4,4,4,4,4]	WT	WT		≤ 0.03	0.08	≤ 0.03	≤ 0.125	1	0.25	0.25

[$]NA: Not available. Allelic profiles acquired from pubMLST: [*AAT1, ACC1, ADP1, MP1b, SYA1, VPS13, ZWF1b*]. Shared allelic markers are highlighted in bold and underscored.
*Trailing phenotype with approximately 50% growth inhibition in the concentration range 0.03-4 µg/ml.
**Trailing phenotype with approximately 50% growth inhibition in the concentration range 0.5-4 µg/ml.