Genotypic markers of azole resistance in clinical *Candida tropicalis* isolates in Singapore

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Background: *Candida tropicalis* is a significant cause of invasive fungal infection and is one of WHO's priority fungal pathogens. Mortality rates of candidaemia are high, compounded by acquired anti-fungal resistance. Notably, increasing fluconazole resistance over time has been reported in different *Candida spp.* (1) Although there are new molecular diagnostic tests that may allow earlier identification of invasive fungal infections, these often have limited capabilities in identifying antifungal resistance. This highlights the need for traditional cultures and susceptibility testing to guide antifungal treatment, and for ongoing monitoring and surveillance. The main limitation of culture and phenotypic susceptibility testing is potentially reduce sensitivity, and turnaround-time from sample collection to availability of results. We performed an investigation to identify potential resistance mutations or other genetic markers for anti-fungal resistance in *Candida tropicalis.*

Methods: Whole-genome-sequencing was performed on de-identified fluconazole-resistant *Candida tropicalis* isolated from blood cultures from two tertiary hospitals in Singapore. Isolates were selected based on laboratory susceptibility data as tested by routine methods (Etest® or Sensititre ®). Isolates tested by Etest were interpreted by European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, while isolates tested by Sensititre were interpreted using Clinical and Laboratory Standards Institute (CLSI) breakpoints.

Results: A total of 125 azole-resistant *Candida tropicalis* isolates were included. One isolate was excluded from WGS analysis due to bacterial contamination. 88.8% (111/124) of isolates had ERG11 resistance mutations identified, with the most common being concurrent Y132F and S154F. The frequency of mutations in decreasing order is as follows: Y132F and S154H (81.98%); Y132F, S154H, and Y257H (7.21%); Y132F (4.50%); S154H (2.70%); Y257H (1.80%); Y257N (0.90%); Y132F and Y257H (0.90%).

All isolates also had elevated minimum-inhibitory-concentration to voriconazole, with majority being in the resistant range (105/111; 94.6%). The MIC distribution of isolates tested by Sensititre were analyzed and stratified by ERG11 mutations (Table 1 and 2). The majority of isolates had marked increase in MICs. Isolates with only Y257H or Y257N mutations had relatively lower MICs, with voriconazole being interpreted as Intermediate

Table 1: Fluconazole MIC distribution stratified by ERG11 mutations

Fluconazole MIC							
ERG11 mutation	**8**	**16**	**32**	**64**	**128**	**256**	**>256**
S154F (n=3)						1	2
Y132F (n=5)			1				4
Y132F, S154F (n=87)	2	1		2	7	25	50
Y132F, S154F, Y257H (n=6)			4	1	1		
Y132F, Y257H (n=1)						1	
Y257H (n=1)	1						
Y257N (n=1)	1						
No ERG11 mutations (n=13)	3	5	1				4

Isolates as tested by Etest were not included in the MIC distribution due to the lower number of isolates (n=7).

Table 2: Voriconazole MIC distribution stratified by ERG11 mutations

Voriconazole MIC							
ERG11 mutation	0.25	0.5	1	2	4	8	>8
S154F (n=3)						1	2
Y132F (n=5)				1			4
Y132F, S154F (n=87)	2		1	2	2	18	62
Y132F, S154F, Y257H (n=6)			1	3	2		
Y132F, Y257H (n=1)						1	
Y257H (n=1)	1						
Y257N (n=1)			1				
No ERG11 mutations (n=13)	1	3	4		1	1	3

MIC interpretation is indicated by shading : Intermediate Resistant

Analysis of the SNQ2 gene which encodes an ATP-binding cassette transporter that is associated with azole drug resistance was performed. The A2977G mutation which is associated with the "clade 4" genotype was detected in 88 isolates (79.3% of all isolates with ERG11 mutations, and 70.4% of all azole-resistant isolates. Analysis of CTRG_05978 gene sequence also identified 88 isolates with genotype IV (ATRA). The A2977G mutation of SNQ2 and CTRG_05978 genotype IV together have been previously reported to be associated with clade 4 resistance clone (2,3).

Conclusions: ERG11 mutations remain as the main mutation that are associated with azole resistance in *Candida tropicalis* isolates in Singapore. Our results also highlight the predominance of the clade4 genotype, occurring int 79.3% of isolates with ERG11 mutations. A multiplex PCR for detection of A2977G in the SNQ2 gene and CTRG_05978 genotype IV sequence in combination can rapidly identify 63.2% of *Candida tropicalis* isolates with azole resistance. An assay capable of identifying mutations in codon 132 of the ERG11 gene can rapidly detect resistance in 84% of isolates. Coverage of codons 154 and 257 further increases detection by 2.4% each.

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