ERG6 and ERG2 are major targets conferring reduced susceptibility to amphotericin B in clinical *Candida glabrata* isolates in Kuwait

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INTRODUCTION

Candida glabrata is the second or third most commonly isolated yeast species causing candidemia and other invasive infections. *C. glabrata* is intrinsically less susceptible to azole antifungal drugs. Recent years have also witnessed increasing reports of breakthrough *C. glabrata* infections in patients receiving systemic echinocandin or polyene treatment. In this study, molecular mechanisms of reduced susceptibility (RS) to amphotericin B (AMB) were investigated in *C. glabrata* strains in Kuwait by sequence analyses of genes involved in ergosterol biosynthesis.

METHODS

(1) A total of 13 *C. glabrata* isolates (8 RS-AMB and 5 AMB-susceptible) were used. (2) Antifungal susceptibility test (AST) was done by Etest and by reference broth microdilution. (3) PCR-sequencing of three (*ERG2*, *ERG6* and *ERG11*) genes was performed by using gene-specific primers. (4) The total cell sterol content was analyzed by gas chromatography-mass spectrometry. (5) Phylogenetic relationship among the isolates was investigated by multilocus sequence typing (MLST).

RESULTS

- The AST data for AMB and other antifungal drugs by the reference broth microdilution method are presented in Table 1.
- AMB-susceptible isolates contained only synonymous mutations in *ERG2*, *ERG6* or *ERG11* and total sterol content of 1 isolate was similar to reference strain (Table 2).
- A nonsynonymous (AGA48AAA, R48K) *ERG6* mutation was found in both RS-AMB and AMB-susceptible isolates.
- Four RS-AMB isolates contained novel nonsense mutations at Trp286/Tyr192/Leu341 and two isolates contained nonsynonymous (V126F or C198F) mutation in *ERG6* and their sterol content were consistent with *ERG6* deficiency.
- Other RS-AMB (n=2) isolates contained novel nonsynonymous (G119S or G122S) *ERG2* mutation and their sterol content were consistent with *ERG2* deficiency.
- Isolate Kw861/13 also contained Y141H + L381M mutations while 7 RS-AMB isolates contained only synonymous mutations in *ERG11*.
- Fingerprinting analysis revealed that all isolates with *ERG6/ERG2/ERG11* mutations belonged to a unique genotype (Table 2).

Table 1. Source of isolation and MIC values of 13 *C. glabrata* isolates for AMB, FLC & ANI by Etest and broth microdilution method

Isolate no.	Clinical specimen	MIC (µg/ml) for AMB by Etest	MIC values (µg/ml) by broth microdilution method for		
			AMB	FLC	ANI
ATCC90030	N. A.	0.094	0.5	4	0.016
Kw600/09	Wound swab	0.032	N. D.	N. D.	N. D.
Kw164/15	Urine	0.38	0.25	16	0.5
Kw383/15	Ascitic fluid	0.19	0.5	2	0.016
Kw590/15	Sputum	0.003	0.25	16	0.016
Kw1421/16	Bone	0.25	0.5	1	0.031
Kw844/10	Urine	1.5	1	2	0.031
Kw861/13	ET aspirate	1	1	>64	0.016
Kw96/15	Urine	2	1	0.5	0.031
Kw1856/15	Urine	1.5	1	0.25	0.016
Kw2516/15	Urine	1.5	1	0.5	0.016
Kw2813/15	Urine	4	1	0.25	0.016
Kw3060/15	Wound swab	1.5	1	1	0.031
Kw3357/15	Urine	2	1	2	0.031

MIC, minimum inhibitory concentration; AMB, amphotericin B; FLC, fluconazole; ANI, anidulafungin; N. A., not available; N. D., not done

CONCLUSIONS

- All isolates with *ERG6/ERG2/ERG11* mutations belong to distinct strains.
- Our data show that *ERG6* and *ERG2* are major targets conferring RS-AMB in clinical *C. glabrata* isolates in Kuwait.

Table 2. Susceptibility to AMB, mutations detected in *ERG2*, *ERG6* and *ERG11* genes, fingerprinting by ITS-*ERG2-ERG6-ERG11* sequence-based profile in 13 *C. glabrata* isolates

Isolate no.	Susceptibility to AMB	Non-synonymous mutations detected in			ITS- <i>ERG2-ERG6-ERG11</i> -based profile
		<i>ERG2</i>	<i>ERG6</i>	<i>ERG11</i>	
ATCC90030 ^a	Susceptible	Reference ^b	Reference ^b	Reference ^b	Reference ^b
Kw600/09	Susceptible	I207V	None	None	Unique
Kw164/15	Susceptible	I207V	None	None	Unique
Kw383/15	Susceptible	I207V	R48K	None	Unique
Kw590/15	Susceptible	I207V	R48K	None	Unique
Kw1421/16	Susceptible	I207V	None	None	Unique
Kw844/10	Resistant	I207V + G122S	None	None	Unique
Kw861/13	Resistant	I207V	Δ Nt. 1021, L341*	Y141H + L381M	Unique
Kw96/15	Resistant	I207V	V126F	None	Unique
Kw1856/15	Resistant	I207V	W286*	None	Unique
Kw2516/15	Resistant	I207V	R48K + C198F	None	Unique
Kw2813/15	Resistant	I207V	Y192*	None	Unique
Kw3060/15	Resistant	I207V + G119S	None	None	Unique
Kw3357/15	Resistant	I207V	W286*	None	Unique

^a*C. glabrata* strain ATCC90030 was used as reference strain for susceptibility to amphotericin B (AMB) and strain CBS138 was used as reference strain for *ERG* gene sequencing