

Gene Expression in Antifungal Resistant and Susceptible Isolates of *Candida albicans*

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SUSCEPTIBILITY assays using white and opaque *Candida albicans* were performed against two antifungals: fluconazole (FLU) and terbinafine (TRB). The microdilution method (NCCLS M27-A2) was used for determination of the MIC₉₀. It was found that FLU and TRB were active antifungal drugs against *C. albicans* either in white or opaque forms but FLU more efficient than TRB.

The expression of five resistance genes was measured by RT-PCR after treatment of *C. albicans* with both FLU and TRB. The results indicated that *Candida* drug resistant gene *CDR1* was expressed in susceptible and resistant isolates. However, *Candida* drug resistant gene *CDR2*, *Candida* multidrug resistant gene *CaMDR1* and ergosterol resistant gene ERG11 were up regulated in only resistant *C. albicans* against FLU and TRB. Fluconazole resistant gene *FLU1* in *C. albicans* not expressed at all antifungal treatments.

Keywords: *Candida albicans*, Antifungal drugs, Resistance genes.

Fluconazole is water-soluble and highly bio-available, permitting both intravenous and oral formulations. It has low binding to plasma proteins and a long plasma half-life (31 hr in adults). Penetration of FLU into the Cerebrospinal fluid (CSF), brain, liver, spleen, and kidneys is excellent. It is excreted unchanged in the urine, making it an excellent agent for the treatment of *Candida* urinary tract infections (Kucers *et al.*, 1997). As with all azoles, FLU is fungistatic. It is usually active against *Candida albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. lusitaniae*. Intrinsic resistance to FLU has been observed in *C. krusei* and *C. glabrata* (Casalino *et al.*, 2004). FLU is the only azole with significant data on use in neonatal populations. The initial neonatal dosing information was developed in a study using FLU as prophylaxis during a *C. parapsilosis* outbreak (Saxen *et al.*, 1993).

Recently, fluconazole-resistant *C. albicans* strains and intrinsically resistant *Candida* species such as *C. glabrata* and *C. krusei* are emerging in immunocompromised patients treated for therapy or prophylaxis (Case *et al.*, 1991; Krcmery and Barnes, 2002).

Terbinafine hydrochloride is a synthetic allylamine antifungal has been developed as a new class of ergosterol biosynthetic inhibitors that are functionally as well as chemically distinct from the other major classes of ergosterol-inhibiting antifungal agents (Ryder *et al.*, 1984; Ryder and Favre, 1997). The mechanism of action of terbinafine involves the specific inhibition of fungal squalene epoxidase, resulting in ergosterol deficiency and accumulation of intracellular squalene, which appears to be identical in dermatophytes, molds, and yeasts (Ryder, 1992; Graminha *et al.*, 2004).

C. albicans resistance to antifungal agent is due to expression of resistance gene(s) associated with antifungal treatment. These genes include *Candida* drug resistance genes (*CDR1* and *CDR2*) they encoding transporters of ATP-binding cassette (ABC) family; multi-drug resistance gene (*CaMDR1*) which coding a major facilitator transporters (MFT) family; fluconazole-resistance genes; (*FLU1*) and (*ERG11*) encoding lanosterol 14- α demethylase (Chen *et al.*, 2010).

The objective of the present study was to assay the susceptibility of *C. albicans* isolates to some antifungals. The upregulation of some resistance genes in *C. albicans* in response to antifungal treatments were also studied.

Material and Methods

Antifungal assay by disk-diffusion susceptibility method

The primary screening of *C. albicans* isolates was carried out by the disk-diffusion method using agar medium (CLSI M44-A), according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2009). The antifungal drugs showing an inhibitory effect on the growth of the tested *Candida* isolates was monitored as an appearance of growth inhibition zone (GIZ).

Estimation of minimum inhibitory concentration (MIC₉₀) using broth microdilution technique (M27-A2)

Liquid RPMI 1640 medium with L-glutamine and 2% glucose but without bicarbonate (Rodriguez-Tudela *et al.*, 1996) was buffered with morpholinepropanesulfonic acid (MOPS) to pH 7 at 25°C, and applied in sterile 96 round-bottom wells microtiter plate made of polypropylene (Nunc®, Roskilde, Denmark) containing different dilutions of antifungal. Thawing was accomplished by setting the inoculated plates in the 4°C refrigerator for 1 hr leaving them at room temperature for another 1hr, and finally placing them in the 35°C incubator for 15 to 30 min to minimize condensation formation on the plate lids. The plate lids also were propped up slightly to allow evaporation of condensate during all three stages of the thawing procedure. MIC₉₀ was defined as the concentration of the antifungal that inhibit 90% of the *C. albicans* growth.

Resistance gene expressions in C. albicans isolates Total RNA isolation

For each isolate, an overnight culture was propagated in fresh yeast extract peptone dextrose broth with constant shaking at 30°C until the mid-logarithmic phase was reached. Total cellular RNA was extracted with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the supplier's instruction. Possible genomic DNA contamination was removed by RNase-free DNase I (Fermentas Life Sciences, Burlington, ON, Canada) treatment. Total RNA concentrations were determined spectrophotometrically at 260nm using a spectrophotometer (SHIMADZU UV-1650PC, Japan): a ratio of A_{260} nm: A_{280} nm of 1.8- 2.0 corresponded to 90-100% pure nucleic acid. Analytical denaturing RNA electrophoresis in TAE agarose gels was carried out based on the method of Masek *et al.* (2005) employing the Tris-acetate-EDTA buffer (TAE) with denaturation of the sample in 50% formamide prior to electrophoresis.

Reverse transcription polymerase chain reaction (RT-PCR) analysis

In order to determine expression genes patterns of *C. albicans* *CDR1*, *CDR2*, *ERG11*, *FLU1* and *CaMDR1* semi-quantitatively, RT-PCR was conducted on total RNA extracted from the most susceptible- and most resistant isolates to fluconazole and terbinafine either white or opaque isolates (Table 1).

TABLE 1. *C. albicans* isolates used for resistance genes expression .

Fluconazole group				Terbinafine group			
Susceptible		Resistant		Susceptible		Resistant	
Opaque C1	White C2	Opaque C3	White C4	Opaque C5	White C6	Opaque C7	White C8

The cDNA for each strain was synthesized using the extracted total RNA with 200 units of RevertAid™ M-MuLV Reverse Transcriptase from the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas Life Sciences) and an oligo (dT) primer [a cDNA synthesis primer (10 μM)] with the dNTP mixture (10 mM). The generated cDNA was then used as a template in PCR reactions. The PCR primers used to amplify and identify the *C. albicans* *CDR1*, *CDR2*, *ERG11*, *FLU1* and *CaMDR1* genes were designed with Primer Premier 5.0 design software (Premier Biosoft International, Palo Alto, CA, USA) and synthesized by BIONEER Inc. (Dalian, Korea). The housekeeping gene *18SrRNA* was used as a control. The nucleotide sequences of the primers are given (Table 2).

TABLE 2. Nucleotide sequences of primers used to amplify the study of resistance genes in 8 *C. albicans* isolates using a semi-quantitative RT-PCR (Semi Q RT-PCR) approach .

Gene	Product length (base pairs)	Direction	Primer sequences
<i>CDR1</i>	93	F	5'-TGTGTACTATCCATCAACCATCAGC-3'
		R	5'-CACCAAAAATAAGCCGTTCTACCA-3'
<i>CDR2</i>	125	F	5'-TGGCAAACAATCCAACAATACA-3'
		R	5'-AATCAAGGGAATAGATGGGTCA-3'
<i>CaMDR1</i>	148	F	5'-TCAGCGGGTCTTTGTTGTATG-3'
		R	5'-GATAATGTTTAGCAAGCCGAGGA-3'
<i>ERG11</i>	134	F	5'-TTTGGTGGTGGTAGACATA-3'
		R	5'-GAACTATAATCAGGGTCAGG-3'
<i>FLU1</i>	92	F	5'-GTGGTAAAATCATCCCCGAAAA-3'
		R	5'-CCAGTCCAACCAAGCCAGA-3'
<i>18SrRNA</i>	150	F	5'-TCTTTCTTGATTTTGTGGGTGG-3'
		R	5'-TCGATAGTCCCTCTAAGAAGTG-3'

ERG11, lanosterol 14 α -demethylase gene; *CDR1*, *Candida* drug resistance 1 gene; *CDR2*, *Candida* drug resistance 2 gene; *CaMDR1*, *Candida* multidrug resistance 1 gene; *FLU1*, fluconazole resistance 1 gene. F, Forward; R: reverse.

In this study different PCR assays were performed in 25 μ L PCR reaction volume. The amplification was performed in a programmable heating block (Primus Thermal Cycler, MWG Biotech, Germany). The PCR conditions for all the genes except *ERG11* was: Initial denaturation of 95°C for 3 min followed by 40 cycles of denaturation at 95°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 1 min. While, the PCR conditions for *ERG11* was: Initial denaturation of 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 45 seconds, annealing at 42°C for 45 seconds, and extension at 72°C for 1 min, followed by final extension at 72°C for 10 min.

Visualization and quantitation of PCR products

After amplification, 10 μ l of each PCR product was mixed with 2 μ l of the orange/Blue 6X loading dye (Promega). Samples were electrophoresed on 1.5% agarose gel in 0.5xTBE buffer containing 0.5 μ g/ml ethidium bromides for 50-60 minutes at 70 volts in a minigel electrophoresis unit using 0.5xTBE as electrophoresis buffer. Amplification products were visualized and photographed using ultraviolet transilluminator. The sizes of the amplification products were compared with either the 100 bp ladder DNA marker that was loaded with the samples simultaneously. Quantitation of RT-PCR produced band intensity, strength, was carried out in Image J software.

Statistical analyses

Duncan test was executed to show homogeneity among the means at ($P < 0.05$). Statistical analyses were performed using SPSS software (version 15; SPSS, Chicago, IL).

Results

1- Antifungal susceptibility assay

Table 3 reveal that the opaque isolates C1 and C3 were more susceptible to FLU than the white ones, C2 and C4. The highly resistant isolate C4 was collected from blood of a male of 2 months age, suffering from immunodeficiency and treated with three types of antibiotics. On the other hand, the highly susceptible isolate C1 was collected from the urine of a male of 2 months age suffering from pynophrosis and treated with two types of antibiotics.

TABLE 3. MIC₉₀ values for FLU against *C. albicans* isolates .

Treatment	MIC ₉₀ (µg/ml)			
	Susceptible		Resistant	
	Opaque C1	White C2	Opaque C3	White C4
FLU	6.5 ±1.1 ^a	15 ±3.0 ^a	76 ±14.0 ^b	200 ±34.0 ^c

Mean±SD, n=3. Across the same row, means with the same letter superscript are homogenous (not significantly different at $P>0.05$), whereas those with different letters are significantly different at $P<0.05$.

Similar trend of result in case of TRB was observed when compared with FLU (Table 4) where the opaque *C. albicans* isolates C5 and C7 were more susceptible than the white ones (C6 and C7) and recorded lower MIC₉₀ against TRB. The highly susceptible opaque isolate C5 to TRB was collected from the blood of a twelve days old female suffering from ectopia vesicae and tetralogy of fallot (TOF), and treated with two types of antibiotics, while the highly resistant white isolate C8 was isolated from blood of a two days old male suffering from skin cancer.

TABLE 4. MIC₉₀ values for TRB against *C. albicans* isolates .

Treatment	MIC ₉₀ (µg/ml)			
	Susceptible		Resistant	
	Opaque C5	White C6	Opaque C7	White C8
TRB	0.6 ±0.2 ^a	5 ±1.0 ^a	56 ±7.0 ^b	450 ±38.0 ^c

Mean±SD, n=3. Across the same row, means with the same letter superscript are homogenous (not significantly different at $P>0.05$), whereas those with different letters are significantly different at $P<0.05$.

Gene expression in C. albicans treated with antifungals

Expression of gene transcripts encoding both fluconazole and terbinafine resistance proteins was analyzed by semi-quantitative RT-PCR (Semi Q RT-PCR) for isolation of total RNA from different *C. albicans* isolates (Fig. 1). The PCR product of the *C. albicans* isolates resulted in a clear band on ethidium bromide stained, UV-transilluminated agarose gel. A single band of PCR product with the expected length on agarose gel electrophoresis also confirmed the specificity of the PCR reactions.

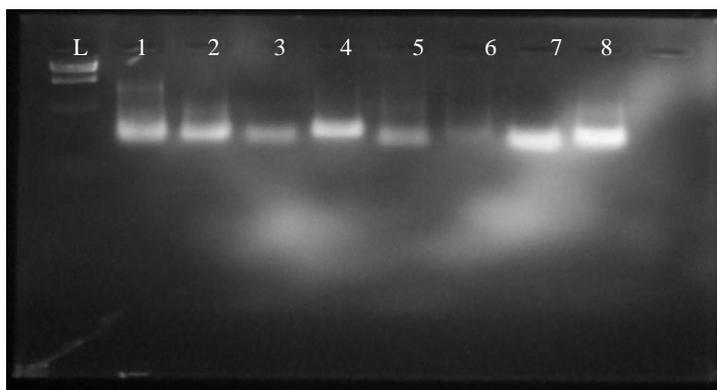


Fig. 1. Analysis of total RNA isolated from *C. albicans* isolates. Lanes: 1, FLU-susceptible opaque isolate C1; 2, FLU-susceptible white isolate C2; 3, FLU-resistant opaque isolate C3; 4, FLU-resistant white isolate C4; 5, TRB-susceptible opaque isolate C5; 6, TRB-susceptible white isolate C6; 7, TRB-resistant opaque isolate C7; 8, TRB-resistant white isolate C8. Total RNA was resolved by denaturing agarose gel electrophoresis in 1X TAE buffer. RNA was dissolved in water (2.5µg/µl) and mixed with deionized formamide to achieve a final concentration (v/v) of 50% formamide. L, RNA ladder (RiboRuler™ RNA ladder high range: 100-6000 bases) (Fermentas).

The expression levels of genes encoding lanosterol 14 α -demethylase (*ERG11*); efflux transporters *Candida* multidrug resistance protein1 (*CaMDR1*); *Candida* drug-resistance *CDR1* and *CDR2* and the major facilitator fluconazole-resistance gene (*FLU1*) implicated in fluconazole-resistance in *C. albicans* were monitored semi-quantitatively by RT-PCR in matched sets of susceptible- and resistant isolates (Fig. 2 a-f). Upregulation folds were calculated based on relative comparison of the resistant with the susceptible strains according to MIC₉₀ of the tested FLU and TRB.

Resistance to FLU

The results showed that *ERG11* transcription level was seen only in the FLU resistant opaque *C. albicans* isolate C3 (Fig. 2-d and Table 5). The *CDR1* efflux gene was up regulated (1.04 and 1.02 fold) in the FLU resistant opaque isolates C3 and FLU resistant white isolate C4, respectively, compared with the susceptible opaque isolates C1 and white isolate C2, respectively. FLU resistant opaque isolate C3 had the highest *CDR1* transcript level (a 1.02 fold increase than *CDR1* in FLU resistant white isolate C4; oppositely, no transcripts were detected for *CaMDR1*, *CDR2*, *ERG11* and *FLU1*, genes in the FLU-susceptible *C. albicans* isolates (C1 & C2) (Fig.2 b & Table 5).

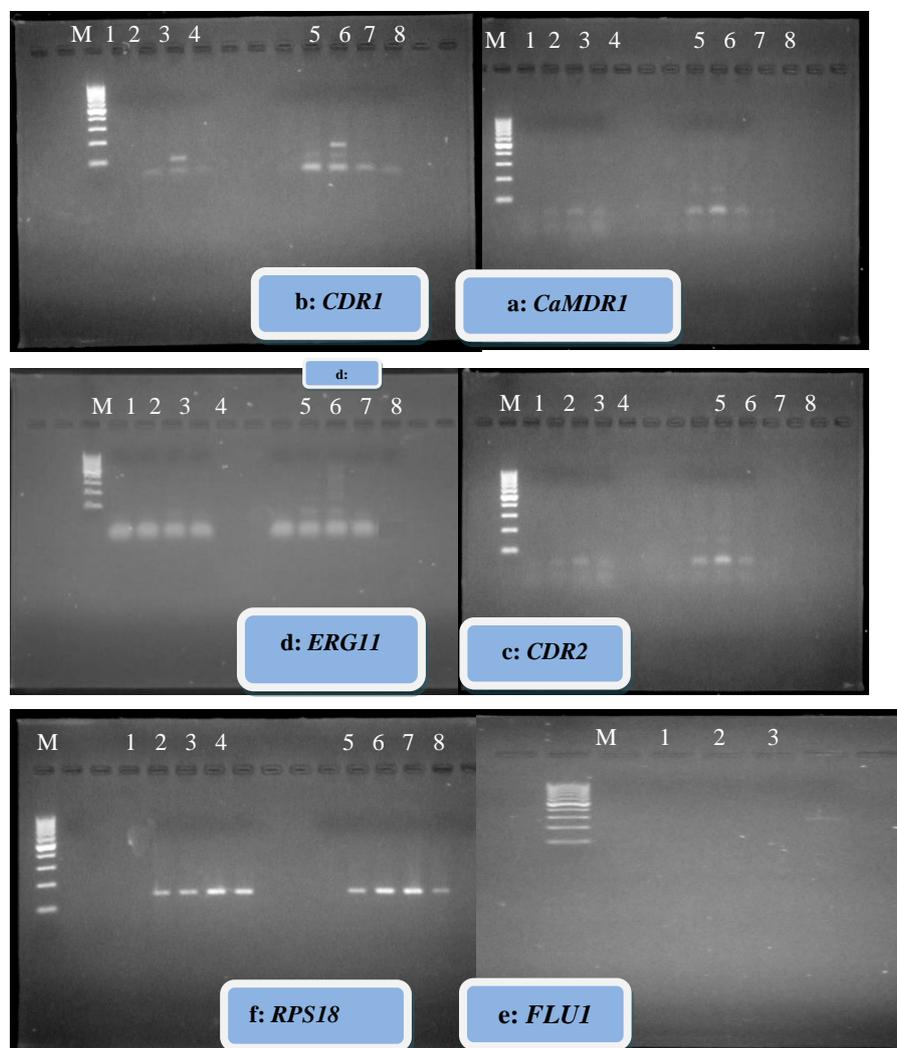


Fig. 2. Semi Q RT-PCR of resistant genes of *C. albicans* of both susceptible and resistant isolates for FLU and TRB. a-e, represented agarose gel electrophoresis of PCR product of different resistant genes, *Ca MDR* (148 bp), *CDR1* (93 bp), *CDR2* (125bp) and *ERG11* (134 bp), *FLU*, (92 bp), and the reference housekeeping gene ribosomal protein S18 (*RPS18*). 5 μ l of PCR products of the represented genes were analyzed on 1.5% agarose gel in 0.5X TBE and stained with ethidium bromide. Lane M: 100bp DNA ladder. Lanes: 1, C1; 2, C2; 3, C3; 4, C4 (fluconazole susceptible- and resistant-isolates) 5, C5; 6,C6; 7, C7; 8, C8 (Terbinafine susceptible-and resistant-isolates).

TABLE 5. Effect of different sensitivity levels of both FLU and TRB on resistance genes expression levels of opaque and white *C. albicans* isolates .

Gene	Fluconazole group						Terbinafine group			
	Susceptible			Resistant			Susceptible		Resistant	
	C1	C2	C3	C4	C5	C6	C7	C8		
<i>CaMDR1</i>	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.663±0.14 ^e	0.484±0.021 ^b		
<i>CDR1</i>	0.805±0.01 ^a	0.801±0.01 ^a	0.844±0.02 ^{ab}	0.821±0.01 ^a	0.935±0.02 ^{bc}	0.765±0.07 ^a	0.972±0.12 ^c	1.126±0.09 ^d		
<i>CDR2</i>	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	2.530±0.16 ^a	2.484±0.37 ^b		
<i>ERG11</i>	0.00±0.00 ^a	0.00±0.00 ^a	2.181±0.01 ^{bc}	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	2.391±0.41 ^d	2.082±0.05 ^b		
<i>FLU1</i> ^o	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	--	--	--	--		

Mean±SD, n=3. Across the same row, means with the same letter(s) superscript are homogenous (not significantly different at $P>0.05$), whereas those with different letters are significantly different at $P<0.05$.

O: opaque isolates, W: white isolates.

^o, *FLU1* is specific to FLU based-isolates and hence not measured in TRB group.

The analysis confirmed that mechanisms of fluconazole-resistance in the four tested isolates of *C. albicans* exhibiting a predominance of over expression of *CDR1* encoding efflux pumps, detected in all tested isolates, while, over expression of the gene that encodes the enzyme 14 α -demethylase (*ERG11*) was detected in 25% of the isolates specifically resistant opaque isolate C3 only.

Resistance to TRB

The *CDR1* efflux gene in the terbinafine-resistant white isolate C8 was up regulated 1.4 fold relative to susceptible white isolate C6. Similarly, 1.04 fold increased in *CDR1* transcript level was observed in resistant opaque isolate C7 relative to the susceptible opaque isolate C5, (Fig.2 b & Table 5). Resistant white isolate C8 had the highest *CDR1* expression level. The resistant opaque isolate C7 had higher up regulation in the *CDR2* transcript level (Fig.2 c) compared with the resistant white isolate C8. The expression of *CaMDR1* (Fig. 2 a) and *ERG11* had maximum expression in resistant opaque isolate C7 followed by white resistant isolate C8. It is worth mentioning that the susceptible white isolate C6 and resistant opaque isolate C5 did not transcript levels for *CaMDR1*, *CDR2* or *ERG11*. However *FLU1* gene not measured in all isolates (Table 5).

The expression of *CDR1* resistance gene by susceptible and resistant *C. albicans* isolates indicated the non selectivity in expressing this gene by *Candida* isolates. The environmental conditions, *Candida* spp. and patient conditions determined whether these genes are functioning or not. Furthermore, the expression of resistant gene *CDR1* by susceptible isolates indicates that it may be expressed but not functioning. *CaMDR1*, *CDR2*, *ERG11* genes were expressed in resistant white and opaque *C. albicans* isolates only (Table 5).

Discussion

Rapid and reliable antifungal susceptibility testing has become particularly important in recent years because of the emergency of antifungal - drug resistance (Asai *et al.*, 1999). Opportunistic fungal infections have dramatically increased in recent years which prompted the pharmaceutical industry to respond with development of several new antifungal agents. Consequently, interest has increased in developing standardized tests to determine antifungal drug susceptibility and in optimizing those tests to accurately predict clinical outcome (Graybill *et al.*, 1998).

In the current study, susceptibility tests and MIC₉₀ determination were carried out on 49 *Candida* isolates (white and opaque forms) of *C. albicans*. Two antifungals with different groups and different mode of actions were tested: fluconazole (FLU) and terbinafine (TRB). Disk-diffusion test (NCCLS M44-A) was used. White-yeast form of *C. albicans* appeared highly susceptible to FLU and TRB. Among the 4 tested isolates of white *C. albicans*, isolates number C2

and C6 were highly susceptible, whereas, isolates number C4 and C8 were the most resistant to FLU and TRB, respectively. Among 4 assayed opaque *C. albicans*, isolate number C1 and C5 were highly susceptible to both FLU and TRB, while isolates number C3 and C7 were highly resistant to FLU and TRB, respectively. In general, the susceptibility tests indicated that opaque-form of *C. albicans* was more susceptible to the tested antifungals than white form.

In spite of FLU widespread use in medical community, many reports observed clinical failure in individuals with candidiasis due to FLU-resistant *C. albicans* strains especially in immunocompromised patients treated for chemotherapy or prophylaxis (Case *et al.*, 1991; Martins *et al.*, 1997; Rangel-Frausto *et al.*, 1999; Krcmery and Barnes, 2002). Pelletier *et al.* (2002) applied NCCLS M27-A method to evaluate the antifungal activity of voriconazole (VRC) and fluconazole (FLU) against 295 *Candida* isolates collected from 189 patients. Forty two isolates had reduced susceptibility to FLU (MIC > 8 mg/l); 83.3% of those had VRC MIC \leq 2mg/l (9 of 11 *C. albicans*, 18 of 19 *C. glabrata*, 6 of 6 *C. krusei*, 2 of 2 *C. Lusitania* and 0.0 of 4 *C. tropicalis*). This suggested that VRC exhibited fungicidal activity against broad range of commonly FLU-resistant species.

In the current study, an experiment was designed to investigate, in the molecular level, the resistance mechanisms of *C. albicans* to FLU and TRB by semi-quantitation of the expression of some resistance genes using RT-PCR. These genes include *Candida* drug-resistance genes (*CDR1* and *CDR2*), the gene encoding lanosterol 14 α -demethylase (*ERG11*), Fluconazole-resistance 1 gene (*FLU1*), and *Candida* multidrug-resistance 1 gene (*CaMDR1*).

It was found that *CDR1* was upregulated by all *Candida* isolates whether it is white or opaque resistant or susceptible to FLU and TRB. This indicated that this gene is over expressed with any antifungal treatment but is functioning in the resistant isolates only. The genes *CDR2* and *CaMDR1* are not expressed in all FLU treatments, while they expressed by resistant isolates (white and opaque) to TRB. *ERG11* was expressed in the two resistant isolates C7 & C8 to TRB and the opaque resistant isolate C3 to FLU. This means that *CaMDR1*, *CDR2* and *ERG11* genes are associated with resistance of *C. albicans* to antifungals. *FLU1* gene was not expressed by *C. albicans* in all cases. It is worth noting that in all susceptible isolates *CDR1* was the only expressed gene.

The most common azole resistance mechanism in *C. albicans* has been reported to be a reduced intracellular accumulation of the drug as a result of reduced influx and / or increased efflux (Ryley *et al.*, 1984; Venkateswarlu *et al.*, 1997). Evidence has been presented that efflux of azole drugs from fungal cells is due to energy-dependent pumps (Vanden Bossche *et al.*, 1992). Correlation between over expression of *CDR1* and *CDR2* genes, which are members of ATP-binding cassette (ABC) super family, and *CaMDR1* gene of Major Facilitator Super family (MSF) and azole resistance by *C. albicans* were detected by several investigators (Sanglard *et al.*, 1995; 1997; White, 1997; Franz *et al.*, 1998). Over

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expression of *CDR1* and *CDR2* without any energy dependent efflux was demonstrated by Sanglard *et al.* (1995). This suggests that although these genes expressed but they are not functioning. High expression of *MDR11* in some FLU-susceptible isolates has been seen by (White *et al.*, 2002; Goldman *et al.*, 2004).

CDR1, *CDR2* and *CaMDR1* are genes proposed to encode cell membrane-associated transporters which act as possible mediator to enhance FLU efflux and they are responsible for the export of several azole drugs (Fling *et al.*, 1991; Sanglard *et al.*, 1995; 1997; White, 1997; Franz *et al.*, 1998). *CDR2* conferred hyper resistance to the antifungal capsfungin, while it enhanced susceptibility to the antifungal micofungin. This indicated that the increased functional expression of *cdr1p* and *cdr2p* didn't give significant candidin resistance in *C. albicans* strains. Chen *et al.* (2010) reported that *CDR1* and *CDR2* were upregulated in all FLU-resistant *C. albicans* isolates whereas only a few isolates showed high expression of *CaMDR1*, *FLU1* and *ERG11* genes compared with control strain. They concluded that over expression of *CDR1* and *CDR2* genes may play role in FLU-resistant *C. albicans*.

In all fungal species, *ERG11* (gene encoding Erg11-p or lanosterol 14 α -demethylase) is an essential gene for ergosterol synthesis. Over expression of *ERG11* causes an increased copy number of the enzyme lanosterol 14 α -demethylase and results in increased ergosterol biosynthesis which reduced the efficiency of the antifungal drugs against *C. albicans* (Holmberg and Stevens, 1999; Perea *et al.*, 2002). The alternation in pathway of ergosterol synthesis in amphotericin B-resistant *C. albicans* involve *ERG11* gene (Liu *et al.*, 1996). Mutation in ergosterol synthesis pathway genes including *ERG11* modifies the site of amphotericin B activity (Kumar and Shakla, 2010), *ERG11* gene over expression result in conformational changes that reduce the effective binding between azoles and their target (Rodriguez-Tudela *et al.*, 1996). Despite this, an investigator has concluded that there is no obvious link between the expression levels of *ERG11* and increased azole resistance (White *et al.*, 2002). Chen *et al.*, (2010) indicated poor correlation between *ERG11* over expression with FLU resistance. Thus, at least four common explanations of the mechanisms of azole resistance in *C. albicans*:

- 1- Alternation of drug target enzymes such as α point mutation in *ERG11* that can affect the affinity between drugs and the target enzyme.
- 2- Overexpression of drug resistance genes in *C. albicans* (*CDR1* & *CDR2*) and the multidrug resistance 1 gene (*CaMDR1*).
- 3- Overexpression of the major facilitator resistance gene *FLU1* which found to increase FLU-resistance by *C. albicans*.

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التعبير الجيني في العزلات الحساسة و المقاومة لمضادات الفطريات في الكانديدا البيكانز

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تم عمل اختبارات الحساسية باستخدام الكانديدا البيكانز البيضاء و الداكنة اللون باستخدام نوعين من مضادات الفطريات هما الفلوكونازول و التريبنافين. و كانت طريقة التخفيف الدقيق قد استخدمت لتقدير قيمة MIC90 .

و قد وجد ان كلا المضادين الفطريين المسخدمين كانا نشطين ضد الكانديدا البيكانز البيضاء و الداكنة اللون، و قد كان الفلوكونازول اكثر كفاءة من التريبنافين.

لقد تم تقديره التعبير عن خمسة جينات مقاومة باستخدام RT-PCR بعد معالجة الكانديدا بكل من المضادين الفطريين. و اشارت النتائج ان جين *CDRI* كان الاكثر تعبيراً في العزلات الحساسة و المقاومة كما كان جين *CDRI* و جين *CaMDRI* و الجين المقاوم للارجوستيرول *ERG11* قد تم التعبير عنهم في السلالات المقاومة للفلوكونازول و التريبنافين فقط. و لم يتم التعبير عن جين *FLU1* المقاوم للفلوكونازول في الكانديدا البيكانز في المعالجات بمضادات الفطريات.