IJPSR (2014), Volume 5, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 March 2014; received in revised form, 25 April 2014; accepted, 28 June 2014; published 01 September 2014

MOLECULAR CHARACTERIZATION OF AZOLE RESISTANCE MECHANISMS IN *CANDIDA ALBICANS* CLINICAL ISOLATES FROM HIV-INFECTED PATIENTS IN INDIA

Desh D. Singh [1], Mayuri Khare [2] and Vinod Singh[*2]

Department of Microbiology, King George Medical University [1], Chowk, Lucknow - 226003, Uttar Pradesh, India.
Department of Microbiology [2], Barkatullah University, Bhopal - 462026, Madhya Pradesh, India.

Keywords:

C. albicans, Azole Drug resistance, Molecular mechanisms, Immunocompromised

Correspondence to Author:
Dr. Vinod Singh

Professor and Head,
Department of Microbiology,
Barkatullah University, Bhopal - 462026, Madhya Pradesh, India.

E-mail: vsingh3@rediffmail.com

ABSTRACT: *Candida albicans* has been the most widely premeditated pathogen in antifungal resistance because of their morbidity and mortality allied with infections in immunocompromised patients. A variety of mechanisms of resistance exist, with the most significant being those due to efflux pumps. The emergence of resistance during therapy in other settings appears uncommon but has been described in settings other than HIV-infected patients. We studied azole-resistant mechanism in 20 isolates of *Candida albicans* from HIV infected patients; these mechanisms include the presence of point mutations in the ERG11 gene and several genes encoding efflux pumps. Using Northern blot analyses, the expression patterns of these genes have been determined during logarithmic and stationary phases of cell growth. Point Mutations and overexpression of ERG11, and several genes encoding efflux pumps, as measured by quantitative real-time reverse transcriptase polymerase chain reaction in 20 clinical isolates of itraconazole resistant *C. albicans* strains. CDR1, CDR2, and MDR1 are expressed early on during logarithmic growth, CDR4 is expressed late during logarithmic growth, and CDR1 is preferentially expressed in stationary-phase cells. MDR1 and CDR mRNAs is transcriptionally overexpressed in the resistant isolate, suggesting that the antifungal drug resistance in this series is associated with the promoter and trans-acting factors of the CDR1, CDR2, and MDR1 genes, while increased mRNA levels of efflux pump genes are commonly associated with azole resistance.

INTRODUCTION: Infectious diseases have been a leading cause of morbidity, disability, and mortality in the world [1]. Azole antifungal agent has been offered for the treatment of infections due to *Candida* since 1990. [2]

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(9).3924-31

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(9).3924-31

Specified the widespread medical knowledge using azole to treat both mucosal and invasive candidiasis, it must be careful one of the preferential agents for the treatment of all types of candidiasis [3]. Clinical resistance of *Candida* to azole has been recognized in much patient care [4].

For *Candida* isolates with reduced susceptibility to the azole antifungal agents, several mechanisms of resistance are known [5]. The primary target for azole antifungal agents is a lanosterol demethylase enzyme, 14alpha-demethylase, which is involved in the conversion of lanosterol to ergosterol [6].

This enzyme is encoded for by the ERG11 gene, previously referred to as CYP51 and ERG16. Azole-induced accumulation of toxic 14- alpha-methyl sterols are also believed to contribute to the action of these agents [7]. The three most commonly proposed mechanisms of azole resistance among Candida species are an alteration of 14- alpha-demethylase decreased intracellular drug accumulation, and loss of function of the enzyme 5, 6-desaturase, which is encoded by the ERG3 gene [8]. Several mechanisms may assist the expression of azole resistance in *Candida sp*.

Since many resistance determinants are often expressed in the same strain and given general lack of availability of serial isolates with changing levels of susceptibility, it is difficult to assess the contribution of each mechanism on the overall level of resistance [9]. The expression or overexpression of drug efflux pumps appears to be the primary mechanism by which Candida sp alter intra-cellular drug accumulation. Adenosine triphosphate-binding cassette transporters (ABCT) and major facilitators (MF) of efflux pumps that contribute to azole resistance among Candida have been identified [10]. Although both functions to remove molecules toxic to cells, efflux pumps from these classes differ in their structure, energy source, and target substrates [11]. Among azole-resistant *Candida sp.*, decreased azole susceptibility correlated with up-regulation of the Candida drug resistance (CDR) genes CDR1 and CDR2 in the ABCT transport family and MDR1 (also referred to as BEN) gene in the MF family [12].

MATERIALS AND METHODS: Twenty clinical isolates of *C. albicans* with azole-resistant were obtained from the different Hospitals from India, Quality control strains of *C. albicans* were also is used in various experiments carried out in the present study. The characteristics of these clinical isolates were confirmed by standard biochemical and microbiological procedures.

Culture of clinical isolates: The strains of *C. albicans* were routinely cultured from single colonies on 10 g of yeast extract, 20 g of peptone, 20 g of dextrose, and 15 g of agar (YEPD) per liter in a conical flask. The medium was autoclaved at 10 psi for 15 min. It was cooled to 45 °C and approximately 15.0 ml of the same dispensed into the sterilized Petri plates from Tarson. The plates were allowed to cool at room temperature for 3 h. Sabouraud dextrose agar plates were inoculated with *C. albicans*, incubated for 96 h in BOD incubator (Thermo Scientific) at 30 °C.

Susceptibility testing: Antifungal susceptibility tests were performed in sterile flat-bottom 96-well microplates, according to the methodology recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]) [13]. Standard compounds of the antifungal drugs were tested: fluconazole (FLC; Sigma-Aldrich) and voriconazole (VRC Sigma-Aldrich), and ketoconazole.

DNA and RNA isolation and real-time RT-PCR: 1×10^6 conidial spore was transferred in a 1.5 ml tube with 700 µl TRIzol (Sigma), followed by addition of 200 ml chloroform. The sample is mixed by inverting the tube for 15 sec, followed by 3-minute incubation at room temperature, centrifugation at 12,000 g for 15 min, and transfer of aqueous phase into a fresh microcentrifuge tube. After that, 500 ml isopropanol was added and centrifuged at 12,000 g for 10 min in cold room. The pellet was washed with 500 ml in 70 % ethanol, centrifuged at max. 7,500 g for 5 min in the cold room dried in air for 10 min and dissolved in 50 ml DEPC-H_2O. To verify RNA integrity, 20 micrograms of RNA were fractionated in 2.2-M formaldehyde, 1.2% agarose gel, stained with ethidium bromide, and visualized with ultraviolet light. The presence of intact 28S and 18S ribosomal RNA bands (semiquantitatively in a 2:1 ratio) was used as a criterion to determine whether the RNA was degraded. RNAse-free DNAse treatment was done.

The absence of DNA contamination after the RNAse-free DNAse treatment was verified by PCR amplification of the resistant gene. cDNA was synthesized by using the Super-Script reverse transcriptase (Gibco, BRL). The Taq- Man R PCR Reagent kit was used for PCR reactions114, 15. The thermal cycling conditions comprised an initial step at 50 °C for 2 min, followed by 10 min at 95 °C, and 40 cycles at 95 °C for 15 sec and 60 °C for 1 min Primer and probe sequences are mentioned in **Table 1**.

TABLE 1: PRIMERS AND PROBE SEQUENCES

Primer/probe	Gen Bank	Accession no.	Sequence (5'–3')
For Light	ACT1	F 1	CCAGCTTTCTACGTTTCC
Cycler	X16377	R1	CTGTAACCACGTTCAGAC
real-time PCR		Probe 1	CGGTATTGTTTTGGATTCTGGTG
		Probe 2	TGGTGTTACTCACGTTGTTCC
	MDR1	*F* 1	GGAGTTTAGGTGCTGT
		R1	CGGTGATGGCTCTCAA
		Probe 1	GCCAGTTGGAGATGGACT
		Probe 2	TTGGTTCATGTGTATCATTTCTGG
	X77589	*F* 1	AAGAGAACCATTACCAGG
	CDR1	R1	AGGAATCGACGGATCAC
		Probe 1	CAAGACCAGCATCTCCATATACTGTAT
		Probe 2	ATTCTTTATGCAAGTGAGGTATGGTG
	CDR2	*F* 1	5'-CACGTCTTTGTCGCAACAGC-3'
		R1	5'-ATGTTGTGACTTGCAGTAGC-3'
		Probe	5'-FAM-CCGTGGTGGGTGGATGCACTGGACAATTCCACGG-Dabcyl-3'
	X1329	*F* 1	TAGAGACGTGATGCTG
	ERG116	R1	AGTATGTTGACCACCCATAA
		Probe 1	AATCTCTGCTACTTATATGAAAGAAATTAAACTGAG
		Probe 2	GAGAACGTGGTGATATTGATCCAAAT
For sequencing	ERG11 ORF	*F* 1	GAAAGGGAATTCAATCG
ERG11 gene	X13296	R1	TGTTAATCCAACTAAGTAAC
		Probe 1	ATTCTTTCCATATTACTTGTCTTC
		Probe 2	AGCAGAAACATCAGATAATTTAG
	Region 2	F2	TATGACGGTTTATTTAGGTCC
		R2	AATATAGTTGAGCAAATGAACG
	Region 3	F3	GCTTCAAGATCTTTATTTGGTG
		R3	TCACCTAAATGTAACAAGAACC
	Region 4	F4	CTTATGGGTGGTCAACATAC
		R4	AGTATCCCATCTAGTTGGATC
	Region 5	F5	GGTTATGCTCATACTAGTGAAAG
		R5	AACAATCAGAACACTGAATCG

Southern Blot Analysis: Genomic DNAs from the susceptible and resistant isolates were prepared as described [Hoffman and Winston, 1987]. Restriction enzyme digestions and Southern blot analyses were performed using standard techniques [16, 17].

Northern Analysis: Logarithmic growth Cells from 24 h cultures grown in YEPD were inoculated in 250 ml of YEPD to a starting concentration of 2 × 10^4 cells/ml. The cultures were grown overnight at 30 °C with agitation. Total RNAs were prepared from cultures of the susceptible and resistant isolates at an optical density at 600 nm (OD600) of 0.1 and at each subsequent doubling time (roughly every 90 min) up to an OD600 of 6.4. Total RNA preparation, gel electrophoresis, Northern blotting, oligonucleotide labelling with polynucleotide kinase, and random priming for radioactive probe preparation were performed with minor modifications according to standard published methods [18, 19].

RESULTS: The minimal inhibitory concentration (MIC) values obtained for 20 *C. albicans* isolates with the three different azoles (fluconazole, itraconazole, and ketoconazole) was performed. The 20 *C. albicans* isolates included 9 that were resistant to itraconazole (MICs ≥64 µg/mL), 6 that were susceptible dose-dependent (SDD for MICs of 16 and 32 µg/mL), and 5 that were susceptible to fluconazole (MICs <8 µg/mL). Regarding itraconazole, one isolate was considered resistant to fluconazole (MIC ≥1 µg/mL), 10 were considered as SDD (MICs of 0.25 and 0.5µg/mL), and 9 were susceptible to fluconazole (MIC > 0.25< 0.5). MIC results show that 12 out 15 isolates SDD or resistant to itraconazole were also considered as SDD (9 for itra and keto) or resistant (1 for itra and 2 for keto) to the other azoles [20].

Otherwise, only 1 out of 5 isolates susceptible to fluconazole exhibited a MIC value compatible with SDD. On Chromagar plates, colonies of the 20 isolates being studied were light green. Data from the API 20C system confirmed that the 20 isolates were *C. albicans*. Total RNA was also prepared from the isolates at 3 days and 8 days of growth. Using these RNAs, Northern blot analysis was performed to examine mRNA levels throughout cell growth for the efflux pumps CDR1 to CDR4 and MDR1 and also the target gene for fluconazole ERG11 [21]. mRNA expression of genes associated with fluconazole, voriconazole, and ketoconazole resistance throughout cell growth.

A susceptible isolate (isolate 1) (MIC 5 1.0 mg/ml) and a resistant isolate (isolate 17) (MIC ≥64 mg/ml) were grown at 30 °C in YEPD, and total RNA was prepared from the culture at an OD600 of 0.1 and each subsequent doubling time to an OD600 of 6.4. For all Northern blot analyses, RNAs were loaded onto the agarose gel so that the visible rRNA bands were equivalent in an amount in all lanes, which ensures equivalent loading of RNAs. Loading based on total RNA concentration can give uneven amounts of RNA because of unequal recovery of small RNAs, including small rRNAs and degraded RNAs, as well as aggregation of RNAs in aqueous solution. We have found that loading based on visible rRNAs is the most accurate. The ACT1 gene was used as a control, as ACT1 is expected to be constitutively expressed under most of the growth conditions.

ACT1 mRNA levels are constant throughout logarithmic growth (usually to an OD600 of 6.0), consistent with the equivalent loading of all gel lanes based on amounts of rRNA. ERG11 mRNA expression consistently showed a small increase in the resistant isolate compared to the susceptible isolate after standardization for ACT1 **Fig. 1**. The ERG11 overexpression in the resistant isolate varied from 1.2- to 2.3- fold. The mRNA levels observed in both growth series (susceptible and resistant isolates) remained roughly equivalent from early to late logarithmic growth.

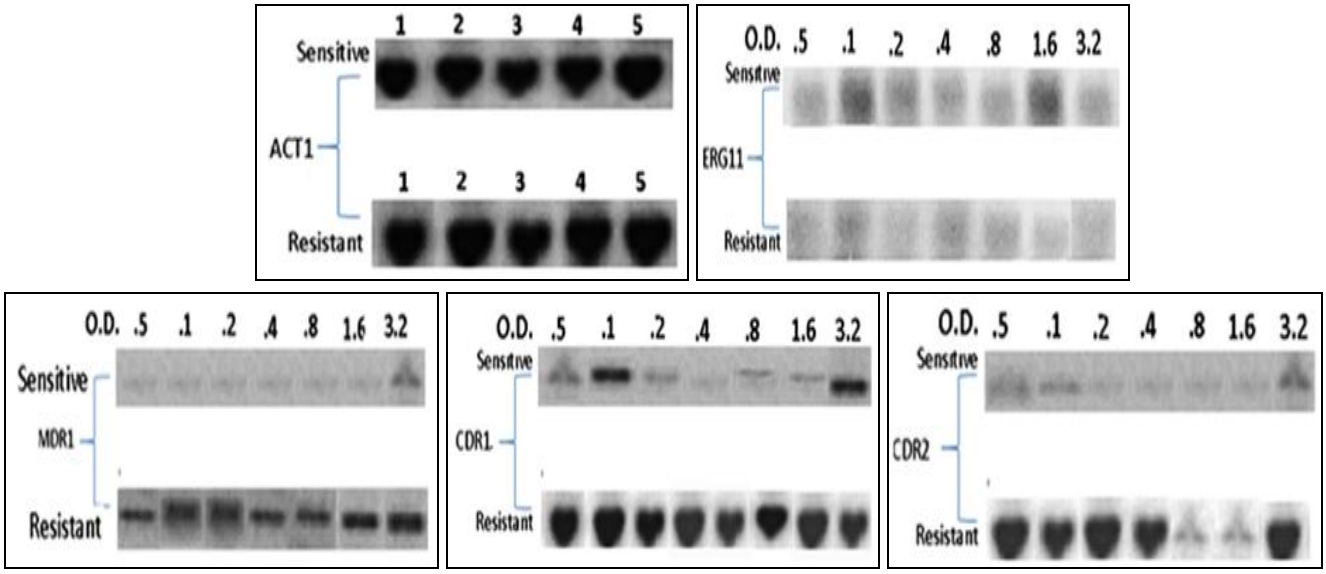

FIG. 1: GENE EXPRESSION DURING LOGARITHMIC GROWTH. TOTAL RNA WAS ISOLATED FROM THE ACTIVE AND RESISTANT ISOLATES AT AN INITIAL OD600 OF 0.5 AND AT EACH DOUBLING TIME (ROUGHLY EVERY 90 MIN) UNTIL THE CELLS REACHED AN OD600 OF 3.2 LANE 1, 2, 3, 4, 5 REPRESNTS 0.5,0.1, 0.2, 0.4, 0.8, 3.2OD, 6.4. NORTHERN BLOTS OF THESE RNAS WERE HYBRIDIZED WITH GENE PROBES FOR ERG11, MDR1, CDR1, CDR2 AND CDR4 (SEE MATERIALS AND METHODS). Each of these blots was simultaneously probed with an oligonucleotide for ACT1.

mRNA levels of the ATP binding cassette transporter genes CDR1 to CDR4 were measured from early to late logarithmic growth (OD600s of 0.1 to 3.2) in both the susceptible and resistant isolates using gene-specific oligonucleotides. mRNA for the CDR1 gene was detected throughout the time course for both the susceptible and resistant isolates [22]. CDR1 was overexpressed in the resistant isolate compared to the susceptible isolate at every time point, with levels of overexpression varying from 2.5 to 7.6. There was an increase in CDR1 expression in the susceptible

isolate at the start of the series-an OD600 of 0.1. There was also a small decrease in CDR1 expression in late log phase in both the susceptible and resistant isolates. Post-diauxic shift phase and stationary phase were determined by repeated monitoring of the growth of the culture and assessing shifts to slower growth (diauxic shift) and eventually, no growth (stationary phase). Low molecular- weight RNA (less than 200 bp) is consistently observed in ethidium bromide-stained gels of RNA prepared at these later time points. RNA levels were monitored by ethidium bromide staining of the rRNA bands in agarose gels, sufficient to obtain qualitative comparisons of the time points [23]. The resistant isolate, there was a consistent reduction in signal for each of the genes when the cells were grown in glycerol or acetate relative to growth in glucose. The largest change in gene expression was a70% reduction in the MDR1 signal for the resistant isolate when the isolate was grown in glycerol or acetate. The expression patterns seen with different carbon sources at an OD600 of the expression patterns were observed during 3 and 8 days of growth of the cells. Total RNA was prepared from the susceptible and resistant isolates at a cell concentration of 6.4 OD600 and 3 and 8 days. Northern blots of these RNAs were hybridized with gene probes for ERG11, MDR1, CDR1, CDR2, and CDR4 **FIG. 2**.

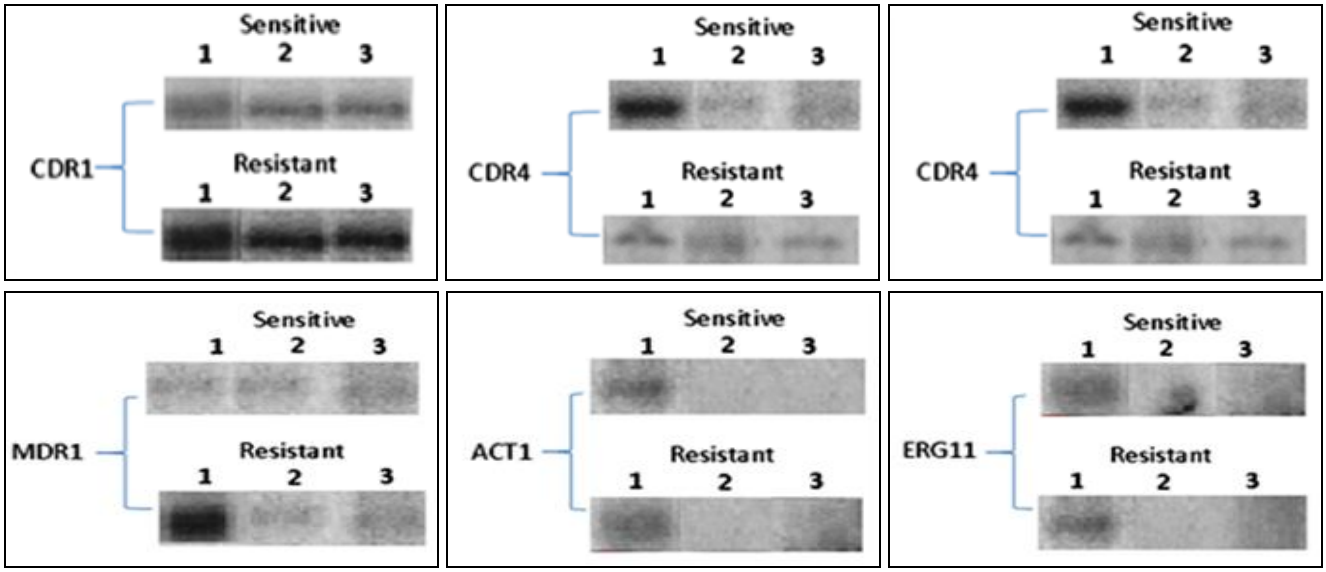

FIG. 2: STATIONARY PHASE GENE EXPRESSION ANALYSIS WERE PERFORMED BY NORTHERN BLOTS. TOTAL RNA WAS ISOLATED FROM SENSTIVE AND RESISTANT ISOLATES AT 6.4OD IN LANE 1 (1 DAY) LANE 2 (3RD DAY) LANE 3 (6TH, DAY) NORTHERN BLOTS OF THESE RNAs WERE HYBRIDIZED WITH GENE PROBES FOR ERG11, MDR1, CDR1, CDR2, AND CDR4

RNAs were loaded so that the visible rRNA bands were approximately equivalent. Relative intensities of gene expression during growth in different carbon sources were also observed. Northern blots were prepared with total RNA from the susceptible and resistant isolates at an OD600 of 1.0 in YEP medium containing glucose (Glu), glycerol (Gly) or acetate (Ace).

The blots of these RNAs were hybridized with gene probes for CDR1, CDR2, MDR1, ERG11, and ACT1. The signals from each blot were quantified using a typhoon imager (GE Healthcare). The intensities were standardized for eac[1]h RNA in the series using the corresponding ACT1 signal for the same lane. The standardized levels were then normalized to the standardized level of the resistant isolate grown in glucose, which was assigned a value of 1. For each gene, the resistant isolate is represented by filled symbols, and the susceptible isolate is represented by open symbols. The relative intensities for each gene are presented on a linear scale with a range of 0 to 1.2 (y-axis, labelled on the left side of the graph). The lines connecting similar data points are presented for interpretation results.

To test for gene amplification of the efflux pumps, Southern blots of genomic DNA from the susceptible isolate and the resistant isolate were

hybridized with MDR1, CDR1, and CDR2. The blots were also hybridized with ACT1 as a control for DNA loading. In this series, the MDR1, CDR1, and CDR2 genes were not amplified in the resistant isolate compared to the susceptible isolate. This eliminates gene amplification as an explanation for increased mRNA levels of these genes

Nuclear run-on analysis for the susceptible and resistant was performed isolates. Southern blots were prepared for DNA (10 mg/sample) from within the coding regions of the gene targets ERG11, MDR1, CDR, ACT1, and a DNA plasmid control. The blots were probed with labeled nuclear run-on RNA. Signal intensities of nuclear RNA were quantified using a typhoon phosphorimager and standardized to the actin intensities. DNA from a pBluescript SK plasmid was used to control for the nonspecific binding of nuclear RNA to random DNA fragments.

The standardized intensities for the susceptible isolate are 0.84, 0.15, 1.56, and 1 for ERG11, MDR1, CDR, and ACT1, respectively. The standardized intensities for the resistant isolate are 0.63, 1.86, 4.12, and 1. Background levels were observed for the DNA controls and the MDR1 signal from the susceptible isolate. Since the MDR1 signal for the susceptible isolate is indistinguishable from background, the MDR1 ratio is a minimal estimate.

DISCUSSION: mRNA levels for ERG11, MDR1, CDR1, and CDR2 are consistently higher in the resistant isolate compared to the susceptible isolate at each time point during cell growth, while overall mRNA levels vary depending upon the stage of cell growth. Two experiments address the possible molecular mechanisms that result in these increased mRNA levels in the resistant isolate: a genomic Southern blot analysis revealed that the genes for CDR1, CDR2, and MDR1 are not amplified, and nuclear run-on analysis demonstrated that one mechanism for increased mRNA levels of MDR1 and CDR is increased mRNA transcription. The Northern analysis revealed that the levels of ERG11 mRNA were slightly increased in the resistant isolate throughout logarithmic growth, and expression in both the susceptible and resistant isolates was constant during growth, correlating with actin mRNA levels. MDR1 mRNA levels in the susceptible isolate were only detectable in early log phase growth, while MDR1 mRNA in the resistant isolate was detected at high levels throughout growth. However, the level of MDR1 mRNA in the resistant isolate varies widely during growth. This variability is likely due to a short half-life of the mRNA, such that variations in the preparation of total RNA from the cells can result in different levels of MDR1 mRNA despite constant levels of ACT1, which is expected to have a relatively long half-life [24, 25]. This variability during growth has been observed in several independent time courses (data not shown). Recently, several mutant alleles of MDR1 were shown to express mRNA at varying levels and were shown to be inducible under several different growth conditions [26, 27].

Expression of CDR1, CDR2, and CDR4 between the susceptible isolate and resistant isolate, were detected by oligonucleotide probes specific for each of the genes. In the susceptible isolate, both CDR1 and CDR2 show expression at early log growth. While CDR2 expression is only detected in early log growth, CDR1 shows expression throughout the logarithmic growth phase. The expression of efflux pumps early in logarithmic growth occurs in cells that have been grown for 16 hrs from very small inoculums. *C. albicans* is likely to grow under a variety of conditions, which do not always include a rich medium containing glucose. Therefore, it was important to examine gene expression after the diauxic shift and during stationary phase.

Most of the genes studied (ACT1, ERG11, MDR1, CDR2, and CDR4) were repressed or down-regulated by 3 days of growth (post-diauxic shift) and were not detectable at 8 days of growth (stationary phase) in both susceptible and resistant cells. The surprising finding was that mRNA was detected for the CDR1 gene at both 3 and 8 days in both the susceptible and resistant isolates. This may be the result of persistent transcription or selective protection from degradation of the CDR1 message.

The nuclear run-on analysis demonstrated that CDR and MDR1 mRNAs were transcribed at higher rates, 2.6-fold and 9.3-fold, respectively, in the resistant isolate than in the susceptible isolate. No increase in transcription for ERG11 was

observed. Previous Northern blot analysis data of RNA prepared at an OD600 of 1.0 showed increased mRNA levels of CDR and MDR1 to be 5-fold and 25-fold, respectively [28].

Our results showed that mRNA levels of ERG11, MDR1, CDR1, and CDR2, which have all been correlated with azole resistance in *C. albicans*, rely on the exact growth phase of the cells. Nuclear run-on analysis has demonstrated that at least one reason for the observed increases of mRNA is an increase in mRNA transcription. However, cell growth and expression of these genes were conducted in vitro. Little is known about the growth environments and growth stages of *C. albicans in-vivo*. Depending upon the type of infection, the yeast may exist in several different states of growth (*i.e.*, hyphal or pseudohyphal), and the cells may be growing exponentially or in a phase resembling the stationary phase. These distinct phases of growth are likely to influence the expression of resistance genes. This is likely to have major implications for azole drug resistance and drug therapy.

CONCLUSION: Molecular tests based on our understanding of resistance mechanisms are unlikely to be sufficient to define clinical resistance in *C. albicans*. It may be important to consider alternate molecular tests that would monitor the general health or status of the fungal cell in the presence of the drug in a shorter time than the present MIC determinations allow. Such tests might be able to accurately identify a resistant fungal isolate quickly, without the need for detailed knowledge about the exact molecular mechanisms of resistance.

ACKNOWLEDGEMENT: This study was solely supported by King George Medical University and Barkatullah University. We would like to extend our thanks to the patients and the pathologists of different hospitals associated with both the Universities for providing clinical samples.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Bennett JE, Izumikawa K and Marr KA: Mechanism of increased fluconazole resistance in *Candida glabrata* during prophylaxis. Antimicrob Agents Chemother 2004; 48: 1773-77.
2. Coste AT, Turner V, Escher F, Morschhauser J and Forche A: A mutation in Tac1p, a transcription factor regulating CDR1 and CDR2, is coupled with the loss of heterozygosity at Chromosome 5 to mediate antifungal resistance in *C. albicans*. Genetics 2006; 172: 2139-56.
3. Cowen LE: The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. Nat Rev Microbiol 2008; 6: 187-98.
4. Goldman GH, Ferreira da Silva ME and Marques ER: Evaluation of fluconazole resistance mechanisms in *Candida albicans* clinical isolates from HIV-infected patients in Brazil. Diagnostic Microbiology and Infectious Disease 2004; 50: 25-32.
5. Cuesta I, Bielza C, Cuenca Estrella M, Larranaga P and Rodriguez Tudela JL: Evaluation by data mining techniques of fluconazole breakpoints established by the Clinical and Laboratory Standards Institute (CLSI), and its comparison with those of the European Committee on Antimicrobial Susceptibility Testing. (EUCAST). Antimicrob. Agents Chemother 2010; 54: 1541-46.
6. Thozee CP, Cronin S, Goj A, Golin J and Ghislain M: Sub-cellular trafficking of the yeast plasma membrane ABC transporter, PDR5, is impaired by a mutation in the N-terminal nucleotide-binding fold. Mol Microbiol 2007; 6: 811-25.
7. Feng LJ, Wan Z, Wang XH, Li RY and Liu W: Relationship between antifungal resistance of fluconazole-resistant Candida albicans and mutations in the ERG11 gene. Med J (Engl) 2010; 5: 544-48.
8. Lyons CN and White TC: Transcriptional analyses of antifungal drug resistance in *Candida albicans*. Antimicro Agents and Chemo 2000; 44: 2296-03.
9. Hamza OJ, Matee MI and Moshi MJ: Species distribution and in vitro antifungal susceptibility of oral yeast isolates from Tanzanian HIV-infected patients with primary and recurrent oropharyngeal candidiasis. BMC Microbiol 2008; 8: 135.
10. Hoffman CS and Winston F: A ten-minute DNA preparation from yeast efficiently release autonomous plasmids for transformation of *Escherichia coli*. Gene 1987; 57: 267-72.
11. Holmes AR, Lin YH and Niimi K: ABC transporter Cdr1p contributes more than Cdr2p does to fluconazole efflux in fluconazole-resistant *Candida albicans* clinical isolates. Antimicrob Agents Chemother 2008; 52: 3851-62.
12. Lee MK, Williams LE, Warnock DW and Beth A: Arthington-Skaggs Drug resistance genes and trailing growth in *Candida albicans* isolates. Journal of Antimicrobial Chemotherapy 2004; 53: 217-24.
13. Lyons CN and White TC: Transcriptional analyses of antifungal drug resistance in *Candida albicans*. Antimicrob Agents Chemother 2000; 44: 2296-03.
14. Makarova NU, Pokrowisky VV and Kravchnko AV: Persistence of oropharyngeal *Candida albicans* strain with reduced susceptibilities to fluconazole among human immunodeficiency virus-seropositive children and adults in a long-term care facility. J Clin Microbiol 2003 41: 1833-37.
15. Maniatis T, Fritsch EF and Sambrook J: Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1982.
16. Mishra BB, Kishore N and Tiwari VK: A novel antifungal anthraquinone from seeds of *Aegle marmelos* Correa family Rutaceae). J. Fitotherepia 2010; 81: 104-07.
17. Mishra BB, Singh DD and Kishore N: Novel antifungal constituents isolated from the seeds of *A. marmelos* Corr. collected in India. Photochemistry 2010; 71: 230-34.

18. Sambrook J, Fritsch EF and Maniatis T: Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Edition 2nd, 1989.
19. Sanglard D, Coste A and Ferrari S: FEMS Yeast Res 2009; 9: 1029-50.
20. Sanglard D and Odds FC: The resistance of *Candida species* to antifungal agents: Molecular mechanisms and clinical consequences. Lancet Infect Dis 2002; 2: 73-85.
21. Selmecki A, Gerami-Nejad M, Paulson C, Forche A and Berman J: An isochromosome confers drug resistance in vivo by amplification of two genes, ERG11 and TAC1. Mol Microbiol 2008; 68: 624-41.
22. Seret ML, Diffels JF, Goffeau A and Baret PV: Combined phylogeny and neighborhood analysis of the evolution of the ABC transporters conferring multiple drug resistance in hemiascomycete yeasts. BMC Genomics 2009; 10: 459.
23. Singh, DD and Singh V: *In-vitro* sensitivity of amphotericin B, itraconazole and fluconazole -resistant against *Candida albicans*. International Journal of Pharmaceutical Science and Research 2011; 2: 1000-04.
24. Swinne D, Watelle M, Van der Flaes M and Nolard N: *In-vitro* activities of voriconazole (UK-109, 496), fluconazole, itraconazole, and amphotericin B against 132 non-albicans bloodstream yeast isolates (CANARIstudy). Mycoses 2004; 47: 77-83.
25. Walker LA, Gow NA and Munro CA: Fungal echinocandin resistance. Fun Genet Biol 2010; 47: 117-12
26. Watamoto T, Samaranayake LP, Egusa HY and Seneviratne HCJ: Transcriptional regulation of drug-resistance genes in *Candida albicans* biofilms in response to antifungal. Journal Med Micro 2011; 60: 1241-47.
27. White TC: Increased mRNA levels of ERG16, CDR, and MDR1 correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. Antimicrob Agents Chemoth 1997; 41: 1482-87.
28. Rogers PD and Barker KS: Genome-wide expression profile analysis reveals co-ordinately regulated genes associated with the stepwise acquisition of azole resistance in *Candida albicans* clinical isolates. Antimicrob Agents Chemother 2003; 47: 1220-27.

How to cite this article:
Singh DD, Khare M and Singh V: Molecular characterization of azole resistance mechanisms in *Candida albicans* clinical isolates from HIV-infected patients in India. Int J Pharm Sci & Res 2014; 5(9): 3924-31.doi: 10.13040/IJPSR.0975-8232.5 (9).3924-31.