Virulence Factors and Azole-Resistant Mechanism of *Candida Tropicalis* Isolated From Candidemia

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Abstract

Background

Virulence factors intensify the pathogenicity of *Candida* species in candidemia. Limited knowledge exists regarding the azole-resistant mechanism and virulence factors of *Candida tropicalis*. Consequently, we aimed to evaluate the virulence factors and the molecular mechanisms of azole resistance among *C. tropicalis* isolated from bloodstream infection.

Materials and methods

Forty-five *C. tropicalis* isolates recovered from candidemia patients were evaluated for virulence factors, including extracellular enzymatic activities, cell surface hydrophobicity (CHS), and biofilm formation. Antifungal susceptibility pattern and expression level of *ERG11*, *UPC2*, *MDR1*, and *CDR1* genes of eight azole resistance *C. tropicalis* isolates were assessed.

Results

The isolates expressed different frequencies of virulence determinants as follows: coagulase 4 (8.9%), phospholipase 4 (8.9 %), proteinase 31 (68.9 %), CSH 43 (95.6 %), esterase 43 (95.6 %), hemolysin 44 (97.8%), and biofilm formation 45 (100%). All the isolates were susceptible to amphotericin B and showed the highest resistance to voriconazole. The high expression of *ERG11* and *UPC2* genes in fluconazole-resistant *C. tropicalis* isolates were observed.

Conclusion

C. tropicalis isolated from candidemia patients extensively displayed capacities for biofilm formation, hemolysis, esterase activity, and hydrophobicity. In addition, the overexpression of *ERG11* and *UPC2* genes can be considered as one of the possible mechanisms of azole resistance.

Introduction:

Nosocomial bloodstream infections (BSIs) caused by *Candida* spp are the fourth and sixth causes of BSIs in the USA and Europe, respectively [1–4]. *C. albicans* is the most common agent causing candidemia, however, the increasing mortality rate due to *non-albicans Candida* (NAC) species reveals their importance [5–7]. *C. tropicalis* is one of the most common agents causing candidemia in some developing countries [8, 9]. A significant link exists between primary colonization and the invasion of infection. The tendency for *Candida* species to cause disseminated invasive infections may be associated with the potential of its virulence factors [10]. Many virulence factors have been represented for candidemia establishment including extracellular secreted enzymes (phospholipase, esterase, coagulase, and proteinase), hemolytic factor, and biofilm formation [11, 12]. These factors can intensify candidemia by *Candida* escape from the immune system to damage the host tissue [13]. The widespread use of antifungals leads to increasing resistance of clinical *C. tropicalis* isolates to some antifungals especially azoles that are one of the reasons for therapeutic failure of candidemia [14, 15]. Among predictors

determining the outcome of candidemia is the susceptibility of *Candida* spp to antifungal agents [1]. Some factors that contributed to azole resistance of *Candida* species contain mutations and/or overexpression of *ERG11* (encoding for cytochrome P450 lanosterol 14a-demethylase), *UPC2* (encoding for the transcription factor of *ERG11* gene), *CDR1* (encoding for efflux protein of ATP binding-cassette (ABC) family), and *MDR1* (encoding for efflux protein of major facilitator superfamily (MFS) family) [16, 17]. Hence, the attention to virulence factors, patterns of antifungal susceptibility, and azole-resistant mechanism of *C. tropicalis* isolates can establish positive management approaches to control candidemia based on the new therapy. This study aimed to evaluate the virulence factors (extracellular enzymatic activities, cell surface hydrophobicity, and biofilm formation), the pattern of antifungal susceptibility and expression of *ERG11*, *UPC2*, *MDR1*, and *CDR1* genes in *C. tropicalis* isolated from candidemia patients in Tehran, Iran.

Materials And Methods

Yeast isolates

During a period of four-year study from December 2016 to December 2020, forty-five *C. tropicalis* isolates were recovered from blood samples of candidemia patients admitted to two tertiary care training hospitals (Imam Khomeini hospital complex and Shariati hospital) in Tehran, Iran. All the isolates were identified using morphological and molecular techniques [18]. Briefly, after sub-culturing yeasts isolated from blood samples on CHROMagar *Candida* plates (Merck, Germany), we confirmed all the isolates using the PCR method. Informed consent was obtained from each patient, person earmarked to accompany the patient or a close family member (often the spouse or parents). This study was approved by the ethics committee of Tarbiat Modares University (IR.TUMS.SPH.REC.1398.286).

Determination of hemolysin production

Hemolytic activity of the *C. tropicalis* isolates was determined using a blood agar assay as described by Manns *et al.* [19]. Seven milliliters of fresh sheep blood was added to 100ml SDA supplemented with glucose 3% (w/v). The final pH of the medium was adjusted to 5.6 ± 0.2 . The prepared media plates were inoculated with $10\mu\text{l}$ suspension (10^6 yeast cells /ml) of both the test and the control *Candida* isolates. The plates were then incubated at 37°C in 5% CO_2 for 48 h. Then, we examined the plates and determined the hemolytic index (Hz value) as the ratio of the diameter of the colony to that of the translucent zone of hemolysis (mm). The interpretation of the results was as follow: high activity ≤ 0.59 ; medium activity 0.6–0.79; low activity 0.8–0.99; negative activity 1 [20]. *C. albicans* (ATCC 14053) was used as positive control while *C. parapsilosis* (ATCC 22019) was used as the negative control. All tests were repeated on two different days.

Determination of proteinase production

The proteinase activity of the isolates was evaluated, according to Staib *et al.* [21]. The *C. tropicalis* suspension was prepared from overnight cultures, of which $10\mu\text{l}$ containing 1×10^6 *Candida* cells /ml was used to inoculate the bovine serum albumin (BSA) agar plate, composed of BSA solution 1%, dextrose 2%, KH_2PO_4 0.1%, MgSO_4 0.05%, and agar 2%. We incubated the plates at 37°C for 72h. Thereafter, we used 20% trichloroacetic acid to fix the plate for 15 min and then stained it with 1.25% amido black for 30 min. We used 15% acetic acid to decolorize the setup before determining the zone (Pz) around the colonies. The classification of proteinase activity was as represented for the hemolysin activity. *C. albicans* (ATCC 14053) was used as positive control while *C. glabrata* (ATCC 90030) was used as the negative control. The assay was performed in duplicate for each sample.

Determination of phospholipase production

The phospholipase activity of the *C. tropicalis* isolates was evaluated according to the technique described previously [22]. Briefly, the egg yolk agar plate was prepared using 13.0g of SDA, 11.7g of NaCl, 0.11g of CaCl₂, and 10% sterile egg yolk (all in 184 ml distilled water). All components were mixed and sterilized before adding the egg yolk. The egg yolk was centrifuged at 500 rpm for 10 min at room temperature and 20 ml of the supernatant was added to the sterilized medium. Ten microliters of *C. tropicalis* suspension containing 10⁶ cells/ml were inoculated onto the egg yolk plates. After drying plates at room temperature, We incubated the plates at 37°C for 48h. The classification of phospholipase activity was as represented for the hemolysin activity. *C. albicans* (ATCC 14053) was used as positive control while *C. parapsilosis* (ATCC 22019) was used as the negative control. The assay was performed in duplicate for each sample.

Determination of esterase activity

Esterase activity of the isolates was evaluated using the Tween 80 opacity test, according to Slifkin *et al.* [23]. To 1000 ml of distilled water, 10g peptone, 5g NaCl, 0.1g CaCl₂, and 15g agar were dissolved; pH adjusted to 6.8 and then autoclaved. Then 5 mL of sterile Tween 80 was added to the media after cooling (at 50 °C) and dispensed into 90 mm plates. Ten microliters of *C. tropicalis* suspension (1x10⁶ cells /ml) were spotted on each plate and incubated for two days at 37°C. The classification of esterase activity was as represented for the hemolysin activity. *C. albicans* (ATCC 14053) was used as a positive control. The assay was performed in duplicate for each sample.

Cellular surface hydrophobicity (CSH)

Candida cells grown overnight at 37°C, were harvested and washed twice with phosphate-buffered saline (PBS) pH = 7.4. *Candida* cell suspension displaying an OD 600 nm equals 1.0 was prepared in PBS (A0); 3 ml of this *Candida* suspension was overlaid by 0.4 ml of the hydrophobic hydrocarbon, xylene (ADWIC, Qalyubiah, Egypt) and incubated in a water bath at 37 °C for 10 min, vortexed for 30 sec, and then returned to the bath for 30 min. The lower aqueous phase was carefully transferred to a clean tube. Any traces were removed by vortexing for 30s. The absorbance at 520 nm was then measured, after fast vortexing. The CSH was expressed as the percentage decrease in the optical density of the test suspension compared with that of the control. The greater the differences in the absorbances, the more hydrophobic were the yeast cells. Each strain was tested in triplicate, on three independent occasions. The CSH was interpreted as follows: 0 = negative, 0.1% -9.99%= low, 10% -19.99% = moderate, >20% = high [20] .

Biofilm assay

C. tropicalis biofilm assay was formed on the bottom of 96-well microtiter plates, and each biofilm was adapted in triplicates as previously described [24]. Briefly, a full loop of freshly picked colonies from SDA medium was inoculated into Sabouraud dextrose broth (SDB; Merck, Germany) medium and was incubated for 18–24 hours at 37°C. After washing 24-hour culture with PBS, cell suspension containing 10⁶ cells /ml was prepared in RPMI 1640 (with L-glutamine and without bicarbonate,) with morpholinepropanesulfonic acid (MOPS, Sigma-Aldrich). A 100 µl of this suspension was inoculated in each well. After incubation at 37°C for 48 hours, wells were carefully washed three times with sterile PBS to remove planktonic cells. Then the biofilm of each well was fixed with 200 µl of 99% (v/v) methanol for 15 min. After washing the plates with dH₂O, 200 µl of 0.1% (v/v) crystal violet (CV) solution was

added into each well for 20 min at room temperature. In the next step, the extra CV was aspirated, and microplates were washed twice with dH₂O; then air-dried. Finally, to dissolve CV bounded to cells, 200 µl of 33% (v/v) acetic acid was added into each well. Biofilm biomass was measured in the wavelength of 595 nm by the Microplate reader (STAT FAX 2100) and the well with *Candida* cell-free-media was considered as a blank and negative control. The cut-off value was calculated according to the average optical densities of negative controls in addition to three standard deviations of negative controls. Biofilm results were interpreted based on the following: categories containing $OD \leq OD_c$ = No biofilm producer, $OD_c < OD \leq 2 \times OD_c$ = Low biofilm producer, $2 \times OD_c < OD \leq 4 \times OD_c$ = Moderate biofilm producer, $4 \times OD_c < OD$ = High biofilm producer. In this study, the cut-off value (OD_c) was 0.243.

Determination of coagulase activity:

Coagulase activities were evaluated as described previously by Yijit *et al.* [25]. Briefly, *C. tropicalis* cells were inoculated into SDB and incubated aerobically at 37 °C for 18–24 hours. A test tube containing 0.5 ml of EDTA rabbit plasma was inoculated with 100µl of the overnight broth and incubated aerobically at 37 °C for different time points containing (2-4-6-24 h). The coagulase activity was interpreted as follows: positive (the presence of platelet clumping) and negative (the absence of platelet clumping). The standard strains of *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 14990) were used as positive, and negative controls, respectively. The assay was performed in duplicate for each sample.

Antifungal susceptibility test

Antifungal susceptibility patterns of the *C. tropicalis* isolates testing was evaluated according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) M27-A3/S4 [26, 28]. We tested the isolates against the following antifungal drugs: fluconazole (FLZ), itraconazole (ITR), voriconazole (VOR), amphotericin B (AMB), caspofungin (CFG), micafungin (MCF), and anidulafungin (AND). Briefly, 100 µl of *Candida* suspension (containing $0.5\text{--}2.5 \times 10^3$ cells/ml) was added to the first ten wells in a row of a 96-wells round bottom microtitre plate, each containing 100 µl of drug diluted in RPMI 1640 (up to the lowest concentration). The eleventh well is positive control containing 100 µl of cell suspension and 100 µl of RPMI; the twelfth well is negative control containing only 200 µl RPMI. We incubated the plates at 37 °C for 24 to 48 h and the results were read as follows: for azoles and echinocandins, the drug concentration at which 50% yeast cells growth was inhibited, and for AMB, at which 100% growth was inhibited were recorded as minimal inhibitory concentration (MIC). The *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019) were used as quality control strains. Interpretation of results was based on the species-specific clinical breakpoint (CBPs) for FLZ, VOR, AND, MCF, and CFG or epidemiological cut-off values (ECVs) for AMB and ITR due to the lack of defined CBPs (Table 2) [27, 28].

Table 1. Primer sequences for gene expression

Name	Primer sequence (5'→ 3')	PCR product length
<i>ERG11-F</i>	TCTGCTTCCACTTCTGCCTG	117
<i>ERG11-R</i>	ATCGTTCAAGTCACCACCCT	
<i>CDR1-F</i>	CCTGAACTGATGCCACGGT	154
<i>CDR1-R</i>	AAGTTTGCCCTGATGGTCGT	
<i>MDR1-F</i>	TGCCTGCTCTATTTACTTGCCT	160
<i>MDR1-R</i>	AGTTCTAGTTGCTGACCATCCA	
<i>UPC.2-F</i>	CAGTGGAACAACAACACAACAA	208
<i>UPC.2-R</i>	TAAATCCCCTAAACCTGAAAGA	
<i>ACT1</i>	TGCTCCAGAAGAACACCCAG	187
<i>ACT1</i>	ACCATCACCAGAATCCAAGACA	

Table 2
Levels of activity of different virulence factors produced by 45 *C. tropicalis* isolates recovered from candidemia patients

Virulence factors (n:39 isolates)	Activity levels			
	High ^a (%)	Medium ^b (%)	Low ^c (%)	Negative ^d (%)
Phospholipase	1(2.2)	1 (2.2)	3 (6.7)	40 (88.9)
Proteinase	6 (13.3)	4 (8.9)	21 (46.7)	15 (33.3)
Esterase	39 (86.7)	3(6.7)	1(2.2)	2(4.4)
Hemolysin	41 (91.1)	2(4.4)	1 (2.2)	1(2.2)
Biofilm	29 (64.4)	15 (33.3)	1 (2.2)	0
CSH	30 (66.7)	9 (20)	4 (8.9)	2(4.4)

Abbreviations: CSH, cell surface hydrophobicity; OD_c, Pz value, the colony diameter divided by diameter of the colony plus zone diameter.

^aPz value, ≤ 0.59; Biofilm, 0.972 < OD; % CSH, >20%.

^bPz value, 0.6-0.79; Biofilm, 0.486 < OD ≤ 0.972; % CSH, 10% -19.99%.

^cPz value, 0.8-0.99; Biofilm, 0.243< OD ≤ 0.486, % CSH, 0.1% and 9.99%

^dPz value, 1; Biofilm, OD ≤ 0.243; % CSH, 0%

RNA extraction, cDNA synthesis, and real-time PCR

The extraction of total RNA from eight *C. tropicalis* clinical isolates treated with FLZ based on the range of susceptibility to FLZ (4 susceptible and 4 resistant isolates) was performed by the RNAX plus kit (Sina clone, Iran). To gain the mass of *C. tropicalis* cells, isolates were cultured in 24-well plates and the total RNA was extracted from cultures of logarithmic phase based on the manufacturer instructions. The determination of RNA concentrations was done by spectrophotometric measurements (Biochrom WPA Biowave II, UK). Extracted RNA was employed to synthesize first-strand complementary DNAs (cDNAs) by the cDNA synthesis kit (Parstous, Mashhad, Iran). The expression levels of *ERG11*, *UPC2*, *MDR1*, and *CDR1* genes were evaluated by real-time PCR. The primer design was performed according to the relevant gene sequences of *C. tropicalis* (Table 1). Real-time PCR was done by Rotor-Gene 3000 real-time PCR system (Applied Biosystem, USA) as previously described [29].

Statistical analysis

All statistical analyses were performed using GraphPad Prism 6.05 software. We used simple frequencies to describe the virulence factors and the antifungal susceptibility profile of the *C. tropicalis* isolates. The correlation between %CSH and biofilm values was determined using Spearman's rank correlation. Continuous variables were compared using the student's t-test. We considered P values ≤ 0.05 to be statistically significant in all tests.

Results:

The relationship between the virulence factors produced and their different level of activities among 45 clinical isolates of *C. tropicalis* was shown in Table 2. Four (8.9%) of the isolates were coagulase-positive, 4(8.9%) were phospholipase-positive, and 31 (68.9%) were proteinase-positive. Whereas all the isolates 45 (100%) evaluated produced biofilm, 44 (98%) produced hemolysin, 43 (95.6%) produced esterase, and 43 (95.6%) showed positive cell surface hydrophobicity (CSH). Table 3 shows the antifungal susceptibility patterns of the 45 *C. tropicalis* clinical isolates. The isolates showed the highest resistance (13.3%) to VOR but all (100%) were sensitive to AMB. There was no significant correlation between CSH and biofilm mass ($P = 0.912$, $r_s = -0.017$). Four FLZ- resistant isolates of *C. tropicalis* revealed overexpression of *UPC2* and especially *ERG11* gene compared with four FLZ susceptible isolates of *C. tropicalis* (Fig. 1). In contrast, expression levels of *CDR1* and *MDR1* genes between 4 FLZ-resistant *C. tropicalis* isolates and the 4 FLZ-susceptible *C. tropicalis* isolates (Fig. 1).

Table 3
Antifungal susceptibility results of 39 *C. tropicalis* isolates recovered from candidemia

Antifungal agents	MIC (µg/ml)		Category (%)				CBPs (µg/ml)				ECV (µg/ml)
	50%	90%	GM	S/WT	SDD/I	R/NWT	S	SDD	I	R	
Fluconazole	0.5	5.6	0.955	75.5	15.5	8.8	≤ 2	4	-	≥ 8	-
Voriconazole	0.125	1	0.137	51.1	22.2	13.3	≤ 0.12	0.25–0.5	-	≥ 1	-
Itraconazole	0.5	2	0.5	93.4	-	6.6	-	-	-	-	0.5
Caspofungin	0.7	0.062	0.056	91.1	2.2	6.6	≤ 0.25	-	0.5	≥ 1	-
Micafungin	0.062	0.35	0.05	95.5	2.2	2.2	≤ 0.25	-	0.5	≥ 1	-
Anidulafungin	0.062	0.25	0.039	97.7	2.2	-	≤ 0.25	-	0.5	≥ 1	-
Amphotericin B	1	1	0.67	100	-	-	-	-	-	-	2

Abbreviations: CBPs, Clinical breakpoint; ECVs, epidemiologic cut-off values; GM, geometric mean; S, susceptible; SDD, susceptible dose-dependent; I, intermediate; R, resistant; WT, wild-type; NWT, non-wild-type.

Discussion:

During recent years, candidemia by NCA species has been considered an emerging problem [18]. In addition, the mortality rate of candidemia due to *C. tropicalis* is high in comparison with other NCA species [30]. The colonization and tissue invasion of *Candida* species are associated with their ability to produce virulence factors including extracellular secreted enzymes and biofilm formation [31]. Although the majority of virulence factor studies are about *C. albicans*, few studies evaluate virulence factors of NAC species. The ability of phospholipases to hydrolyze at least one ester linkage in glycerophospholipids of cell membrane leads to the invasion of host mucosal epithelia [32]. In our study, 8.9 % of *C. tropicalis* isolates produced phospholipase. Similarly, other studies reported that phospholipase activity of *C. tropicalis* isolates recovered from blood samples was low or undetectable [2, 33–35]. We also reported that most phospholipase-undetectable *C. tropicalis* isolates had the production capacity of esterase as carboxyl ester hydrolase. This is in agreement with Sriphannam *et al.*, and Atalay *et al.*'s studies [33, 36]. Proteinases by degrading proteins associated with the host immune system such as albumin, mucin, antibodies, complement, and interleukins can protect *Candida* species from host defense [13]. In this study, low and negative proteinase activities were observed in more than 70% *C. tropicalis* isolates. Similarly, in other previous studies, the majority of *C. tropicalis* isolates recovered from blood exhibited low or undetectable proteinase activity [2, 33, 36]. The iron absorption by the activity of hemolysin is one of the critical factors for the survival and growth of pathogenic microorganisms in the host [37]. Our findings demonstrated that 44 (97.8%) *C. tropicalis* isolates produced hemolytic factor with strong activity in 41 (91.1%) of isolates that corroborates Canel *et al.* study [2]. Besides, Franca *et al.* compared the hemolytic activity of 14 *C. tropicalis* and 34 *C. parapsilosis* isolates with reference to anatomic sites and found that *C. tropicalis* isolates from blood had statistically higher

hemolytic activity ($P < 0.05$) than *C. parapsilosis* [38]. Coagulase activity in *Candida* spp. is a virulence factor that facilitates spread and its roles in pathogenicity need not be overemphasized. The low percentage of coagulase activity in this study coincided with Mushi1 MF *et al.* and Deorukhkar *et al.* that indicated coagulase activity of *C. tropicalis* strains isolated from different specimens was low or undetectable [34, 39]. In contrast, Rodrigues *et al.* reported that coagulase activity of *C. tropicalis* isolates was high (83%) [40]. The possible reasons for this contradiction can be attributed to the limited sample size, and/ or innate biological alterations in tested isolates. Biofilm formation is another virulence factor that helps fungal cells to escape the host immune system [41]. In the current study, all the evaluated isolates produced biofilm. Similarly, the studies of Sachin *et al.* and Sriphannam *et al.* revealed that the majority of *C. tropicalis* blood isolates produce biofilm [32, 36]. Also, Negri *et al.* study indicated that the ability of *C. tropicalis* clinical strains to form invitro biofilm on silicon is extensive [42, 43]. CSH, a marker related to the composition of cell wall, and biofilm formation ability are usually evaluated together [7]. A previous study that evaluated the virulence profile of 184 *Candida* species showed *C. tropicalis* strains, among the different *Candida* species, with the most biofilm biomass had not any correlation with its high hydrophobicity [7]. This study agrees with our results in which high hydrophobicity (91%) of *C. tropicalis* isolates had no correlation with their biofilm mass. Among the attributes of *Candida* species, hydrophobicity and biofilm formation protect the yeast cells from environmental stresses and human host defenses with a subsequent decrease in susceptibility to antifungal agents leading to treatment failures [44]. Moreover, susceptibility to antifungal agents, among other factors, determines the outcome of established candidemia. In the current study, we evaluated the susceptibility of the *C. tropicalis* clinical isolates against the three classes of antifungal agents. The highest resistance rate of our isolates associated with VOR (13%) is consistent with previous studies in India and Iran [18, 35]. Generally, previous studies reported that echinocandins have a reasonable impact on *C. tropicalis* isolates while our isolates revealed low resistance to echinocandin [8, 45]. Consistent with our findings, the studies of Khan *et al.*, Bessetti *et al.*, and Deorukhkar *et al.* revealed that all *C. tropicalis* blood isolates were susceptible to amphotericin B [1, 34, 46]. Available data regarding the azole-resistant mechanism of *C. tropicalis* strains in comparison with other species of *Candida* is not enough. In our study, the expression of the *ERG11* gene in FLZ-resistant *C. tropicalis* isolates was higher than FLZ-susceptible *C. tropicalis* isolates, as found in other studies conducted in France and China [16, 17, 47]. Furthermore, Choi *et al.* reported that high expression level of the *ERG11* gene was observed in FLZ- non and -less susceptible *C. tropicalis* isolates compared with FLZ-susceptible [48]. Upregulation of *ERG* genes can attribute to enhanced expression of *UPC2* gene as transcription factor [17]. In the present study, the *UPC2* expression in FLZ-resistant *C. tropicalis* isolates was higher than FLZ-susceptible that supports Jiang *et al.* study [17]. Therefore, it can be proposed that *ERG11* and *UPC2* genes have an effective impact on the molecular mechanisms behind FLZ-resistant *C. tropicalis* isolates. In contrast, our study did not find any difference in the expression levels of *CDR1* and *MDR1* genes between resistant and susceptible *C. tropicalis* isolates, an observation that corroborates Jiang *et al.* study [16]. However, Barchiesi *et al.* revealed that the resistance of *C. tropicalis* isolates was the connection to overexpression of *CDR1* and *MDR1* genes [49] that one of explanations for this controversy is the mechanism of acquisition of resistance to FLZ in *C. tropicalis* isolate *in vitro* can differ from our clinical isolates. Furthermore, collected isolates of *C. tropicalis* may be heterogeneous.

In conclusion, our findings provide experimental data on production frequency of virulence factors contributing *C. tropicalis* pathogenesis. All the isolates were susceptible to AMB. We have also indicated the involvement of *ERG11*, and *UPC2* genes in the mechanism of FLZ resistance in *C. tropicalis* isolates. This work has some limitations, such as our inability to investigate point mutations of involved genes in *C. tropicalis* isolates as an alternative mechanism of azole resistance, and virulence factors based on *in vivo* and molecular methods.

Declarations

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Disclosure of interest:

The authors declare that they have no competing interests.

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Figures

Figure 1

The pattern of ERG11, CDR1, MDR1, and UPC2 expression in groups of FLZ-susceptible and -resistant *C. tropicalis* isolates. Each gene was quantified using $-\Delta\Delta CT$ by the control gene (ACT1). Error bars indicate standard deviation.