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Original Article

Vitek 2 system for identification and susceptibility testing of *Candida* species with detection of fluconazole resistant genes

Microbiology

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ABSTRACT

Background: *Candida species (Candida spp)* has been increasingly emerging nosocomial pathogens with increasing treatment failure, so it is important to isolate, identify the species and check the sensitivity pattern. Fluconazole resistance is linked to multiple molecular pathways demonstrated by *Candida spp*.

Objective: To determine the frequency of Candida spp. isolated from a variety of clinical samples, antifungal susceptibility patterns and detection of fluconazole-resistant genes expression pattern.

Methodology: A cross-sectional study was performed on 300 various clinical samples in a hospital setting from patients attending Outpatient and Inpatient departments of Tanta University hospitals. Several candida spp. was identified at species level by using conventional tests, and *VITEK 2* compact system. Antifungal susceptibility testing was carried out using *VITEK 2* compact automated system and disk diffusion method followed by detection of gene expression pattern for *ERG11*, and efflux pumps by Real-time- PCR in *candida species* resistant to fluconazole.

Results: From 300 clinical samples, 51 different species of candida were identified. *Non albicans candida (NAC)* were more prevalent (56.9%) than *C. albicans* (43.1%). Overall, the isolated *candida species* 20 (39.2%) were resistant to fluconazole. Regarding to gene expression pattern *Ergosterol (ERG11), Candida drug resistance (CDR1, CDR2),* Pleiotropic drug resistance *(PDR1)* in *C. glabrata*, there was a statistically significant difference in the *ERG11, CDR1* expression levels only in resistant isolates compared to sensitive isolates. while analysis of gene expression of *C. auris* efflux pump *(CDR1, CDR2),* Multidrug resistance (*MDR1) and ERG11*, revealed statistically significant differences in all tested genes.

Conclusion: There was increase in the frequency of *non albicans Candida (NAC)* and emergence of fluconazole resistant species; *C. auris* emerged during pandemic of COVID-19 and they significantly exhibit expression levels higher than *C. glabrata*.

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Keywords: VITEK 2 system; *non albicans Candida*; fluconazole resistance; RT-PCR; *Candida auris*.

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INTRODUCTION

Globally, fungal infections are most frequently caused by the yeast of genus *Candida*. It is also unique among mycotic pathogens as it causes a wide spectrum of clinical symptoms, ranging from mucocutaneous infections to potentially fatal systemic infections. These symptoms can be linked to multiple risk factors, such as use of invasive devices and prolonged use of antibiotics [1].

Candida albicans is the primary cause of candidiasis in most cases. These days, several research have suggested that *non-albicans Candida species (NAC)* such as *Candida parapsilosis, Candida tropicalis, Candida glabrata, and Candida krusei* are a major source of *Candida* infections. Furthermore, *C. auris* is a newly discovered, multidrug-resistant fungus that has been linked to invasive infection outbreaks in medical facilities all over the world [2].

Although the patterns of susceptibility to antifungal drugs vary throughout *Candida* species, certain *NAC* spp. show inherent resistance to specific antifungal medications. For a correct diagnosis and optimal therapy selection, accurate identification of isolates at

species level, and antifungal sensitivity are necessary [3].

Azoles target a particular step in the manufacture of ergosterol, which encodes the *ERG11* gene encodes and catalyzes lanosterol 14α-demethylation. Fluconazole is the most commonly used triazole in clinical settings, because of its excellent oral availability and patient acceptability. Azoles are fungistatic to *Candida spp.* therefore, the long term and repeated courses of therapy have produced clinical isolates resistant to azole which has led to treatment failure and elevated patient mortality [4]**.** Moreover, two families of membrane-associated efflux pumps; (The ATP-binding cassette (ABC) superfamily and the major facilitator superfamily (MFS)) have been found to be overexpressed in resistant isolates of *Candida spp*., these mechanisms are linked to fluconazole resistance. Candida Drug Resistance Genes (*CDR1* and *CDR2*) are the two main ABC transporter genes and *MDR1* (Multidrug resistance1) as a member of MFS**.** Overexpression of *ERG11* and point mutation within *ERG11* has also been shown to be contributors to *Candida spp.* resistance [5]. Comprehending resistance at the molecular level is crucial for formulating tactics to address resistance and justifying the creation of novel antifungals and target-based molecular techniques [5]. So, the present study was designed to detect the prevalence of *Candida spp.* isolated from various clinical samples, antifungal susceptibility patterns and detection of fluconazole-resistant genes expression pattern.

PATIENT AND METHODS

Study design and population:

A cross-sectional study was carried out on 300 patients attending Outpatient and Inpatient departments of Tanta University hospitals during the period between May 2021 and March 2022. This study was conducted in compliance with Helseinki's declaration, and the approval of the ethics committee of Al-Azhar University of medicine for girls, (the protocol number R. 202003224). The laboratory work was done in Microbiology Department of Faculty of Medicine for Girls, Al- Azhar University and Al- Zahraa hospital. Verbal consent was taken from every patient to participate in the study.

A complete history was taken with demographic details including (age, sex, name, presenting complaints, signs, symptoms, presence of predisposing factors and treatment).

Inclusion criteria:

Individuals with clinically suspected candidiasis of both sexes and all age groups who visit outpatient and inpatient departments were included as the following:

- Intensive care units.
- Patients with excessive antibiotic intake.
- Individuals with diabetes have a higher chance of developing fungal infections.
- Women with symptoms of vaginitis.
- COVID 19 infection.
- Infants between 0 day and 18 months of age suffering from Diaper Dermatitis.

Exclusion criteria:

- Patients receiving antifungal medication.
- Patients refused to participate.

1. Isolation of *Candida spp*.:

– *Candida spp.* isolated from different clinical specimens includes blood, sputum, bronchoalveolar lavage (BAL), wound swabs, skin swabs, urine samples from catheterized patients and vaginal swabs were processed in the department of Microbiology.

– The presumed *Candida* colonies were recognized by wet film, Gram stain, colony morphology on Sabouraud's Dextrose Agar (SDA) (Himedia, Mumbai, India) supplied with chloramphenicol, and Urease test [3].

– Pure isolates were stored in Brain heart infusion broth (Himedia, Mumbai, India) containing glycerol with 10% conc. (Sigma-Aldrich, South Africa) at – 80°C until further use.

– Quality control strains of *C. albicans* American Type Culture Collection (ATCC) 10231 and *C. glabrata* (ATCC) 2001 obtained from Central lab of Faculty of Science for Girls, Al- Azhar University were used in all tests.

2. Speciation of *Candida*:

It was carried out by assessing the germ tube test (GTT), colony color on Hicrome ™ Candida Differential agar (Himedia, Mumbai, India), growth at 42- 45°C for discrimination between *C. albicans* and *C. dubliniesis*, and sugar assimilation profiles obtained by Vitek-2 Compact System (bioMérieux Diagnostics, Lyon, France) in Al-Zahraa Hospital University using yeast identification (YST)- 21343 cards [1].

3. Antifungal susceptibility testing:

a. **Using AST-YS08 cards and the VITEK 2 compact automated system** (bioMérieux, France), antifungal sensitivity patterns for these antifungal drugs (amphotericin B, flucytosine, fluconazole, voriconazole, caspofungin, and micafungin) were assessed for all *Candida* isolates in compliance with manufacturer guidelines. In short, The DensiChek (Biomerieux, France) equipment was used to adjust the 2.0 McFarland standards (acceptable range of 1.8 to 2.2) before the *Candida* cultures were placed into glass tubes containing 3 mL of 10% saline. Once the culture suspension was ready, the loaded cassettes were inserted into the Vitek 2 instrument and the Vitek 2 ID and AST-YST cards were immediately filled. The advanced expert system (AES) version 7.0 software was utilized to assess the outcomes generated by the Vitek 2 system. The cards were subjected to an 18-hour incubation period at 35.5 °C. Data were gathered every 15 minutes during this time to ensure definite identification and the minimum inhibitory concentration (MIC)

breakpoint values, which needed to categorize *Candida spp.* as susceptible, intermediate, or resistant to each tested drug.

b. **Disk diffusion method:** It was done for *Candida spp.* not detected by Vitek- 2 compact system (*C. glabrata, C. auris*) using fluconazole discs (25 µg) (Himedia, Mumbai, India). In short, A sterile 0.85% saline solution containing an inoculum with a turbidity of 0.5 McFarland level for every isolate was used to inoculate Mueller-Hinton agar (MHA) (Himedia, Mumbai, India). The MHA was supplemented with 2% glucose to provide suitable fungal growth and 0.5 µg/ml methylene blue dye to enhance zone edge definition, and the inoculum was incubated for 24 hours at 35°C. Using CLSI interpretive breakpoints, inhibition zones were interpreted [6].

4. Quantification of genes expression in fluconazole resistant strains by RT- PCR:

- RNA extraction: Total RNA was extracted from *C. auris* and *C. glabrata* as they were the most common isolated species resistant to fluconazole using ABT- Total RNA Mini Extraction Kit (Applied Biotechnology, Cat # ABT002, Egypt) according to the manufacturer's instructions. Briefly, Strains were grown on SDA (Himedia, India) at 37°C for 24 h, 1ml of the suspension was centrifuged at 2000 rpm for 2 min to pellet the cell, then homogenized in 700 ul of RNA lysis buffer, leave the sample for 5 minutes at room temperature, then mixed by Vortex, After that, 0.2 ml of chloroform was added then mixed by Vortex, tubes were centrifuged at 12,000 rpm for 5 minutes at room temperature, then the upper aqueous phase (containing RNA) was transferred to a microcentrifuge tube, 700 ul of 70% ethanol was added, mix by inversion for 3 times, then, transfer the mixture and precipitate to a Spin-column, followed by centrifugation at 12,000 rpm for 30 second at room temperature. After that, the supernatant was discarded, then 500 µl washing buffer was added, followed by centrifugation at 12,000 rpm for 30s, the flow through discarded and repeated this step. An RNase-free Eppendorf tube was used to elute the RNA, and a Nanodrop spectrophotometer (NanoDrop 2000C, ThermoFisher, USA) was used to determine the RNA's concentration and purity.
- Reverse transcription: All RNA samples were transformed into cDNA using cDNA synthesis Kit (Applied Biotechnology, Co. Ltd, Egypt) according to manufacturers' instructions. RT reaction mix was done as the following: 0.2 – 2 ul RNA Template, 10 ul RT mix and nuclease free water up to 20 ul. The reaction occurred in a thermal cycler (T100, Bio – RAD, USA) with a single cycle of amplification and three different incubation times: 5 minutes at 25°C, 15 minutes at 45°C, and 5 minutes at 85°C. Every sample analyzed was transcribed using the same conditions for the reverse transcription reaction.
- RT- qPCR analysis: Using Real-time PCR, the expression levels of fluconazole-resistant genes *(CgCDR1, CgCDR2, CgPDR1, CgERG11)* in *C. glabrate* and *(CDR1, CDR2, MDR1, ERG11)* in *C. auris*, in addition to the housekeeping gene (β actin used as a normalizing gene) were determined. The primer BLAST tool (https : // www . ncbi . nlm . nih . gov / tools / primer-blast**)** was utilized to test the primers which listed in table (1, 2). Gene expression levels were measured in a 20 µl PCR reaction mixture containing 10 ul of SYBR Green Master mix (Applied Biotechnology, Co. Ltd, Egypt), 1 µl of each primer solution (10 µM), 5 µl of cDNA sample, and water to bring the volume up to 20 µl using the Step One Plus ™ Real-Time PCR Systems (Applied Biosystems, USA).

The used thermal cycling conditions were 95◦C for 4 minutes, followed by 40 cycles of 15 s at 95◦C and 30 s at 60˚C, and melting curve analysis was done. Clinical isolates susceptible to fluconazole used as a calibrator isolate in the study of gene expression. The value of relative quantification (RQ) of the tested genes in relation to control strains were determined by ΔΔ CT method.

Table (1): Primers for quantitative Real-time PCR analysis of target gene expression in *C. auris* [7,8].

Gene	Primer (5′→3′)
ACT1-F	5′- GAAGGAGATCACTGCTTTAGCC -3′
ACT1-R	5′- GAGCCACCAATCCACACAG -3′
CDR1-F	5′- GAAATCTTGCACTTCCAGCCC -3′
CDR1-R	5′- CATCAAGCAAGTAGCCACCG -3′
CDR2-F	5′- GTCAACGGTAGCTGTGTG -3′
CDR2-R	5′- GTCCCTCCACCGAGTATGG -3′
MDR1-F	5′- GAAGTATGATGGCGGGTG -3′
MDR1-R	5′- CCCAAGAGAGACGAGCCC -3′
ERG11 -F	5′- CGCTAAGCTTGCGGATGTTT- 3′
ERG11 -R	5′-ACTGGAGTGGTCAAGTGGGAAT- 3′

Table (2): Primers for quantitative Real-time PCR analysis of target gene expression in *C. glabrate* [5].

Gene	Primer (5′→3′)
CgACT1-F	5′-TTGACAACGGTTCCGGTATG-3′
CgACT1-R	5′-CCGCATTCCGTAGTTCTAAG-3′
CgERG11-F	5′- CCCACCTACTGACTTCACCT- 3′
CgERG11-R	5′- GATCTTAGCAGGGGCAGTTG- 3′
CgCDR1-F	5′- CATACAAGAAACACCAAAGTCGGT- 3′
CgCDR1-R	5′-GAGACACGCTAACGTTCACCAC-3′
CgCDR2-F	5′-GTGCTTTATGAAGGCTACCAGATT-3′
CgCDR2-R	5′-TCTTAGGACAGAAGTA ACCCATCT-3′
CgPDR1-F	5′-TTTGACT CTGTTATGAGCGATTACG-3′
CgPDR1-R	5′-TTCGGATTTTTCTGTGACAATGG-3′

Statistical analysis

Qualitative data were presented by numbers (No.) and percentages (%); quantitative data: presented by mean and standard deviation. Independent sample t test (t) was used for quantitative variables. Using Program for Social Science (SPSS) version 24.0 (SPSS Inc., Chicago, Illinois, USA). p-value ≤ 0.05 is considered significant.

RESULTS

A total of 51 *Candida* spp. was separated from 300 clinical samples. It was found that candidiasis is more in female (64.7%) than male (35.3%), the patient mean age was (43.3 ± 17.1) ranged from 1 - 67 years. Patients with different risk factors includes 10 (19.6 %) diabetic patients, 7 (13.7 %) patients with chronic diseases, 5 (9.9%) infants with napkin dermatitis, 16 (31.4 %) patients suffering from vaginitis, 5 (9.9 %) patients with COVID 19, 8 (15.7 %) ICU admitted patients on antibiotic therapy as shown in table (3).

From results of different methods used in speciation of *Candida* isolates (table 4), *NAC* were more prevalent 29/51 (56.9%) than *C. albicans* 22/51 (43.1%). Among *NAC*, there were 5 (9.8%) *C. tropicalis*, 7 (13.7%) *C. auris*, 2 (3.9%) *C. krusi*, 12 (23.5%) *C. glabrata*, 1 (1.96%) *C. famata*, 1 (1.96%) *C. gluilliermondii* and 1 (1.96%) *C. dubliniensis* in the isolated strains (figure 1).

Regarding antifungal susceptibility patterns obtained by Vitek- 2 compact automated system, and disc diffusion method for fluconazole; we found that out of 51 *Candida* isolates, there were 20 islolates resistant to fluconazole. All of them belong to NAC as the following: 8 (40 %) isolates of *C. glabrata*, 7 (35 %) isolates of *C. auris*, 2 (10%) isolates of *C. krusie* and one (5 %) isolate of *C. famata*, *C. guilliermondii*, *C. dubliniensis* for each (table 5).

Expression of fluconazole resistance genes in *Candida glabrata*, there was statistically significant difference in the *ERG11*, *CDR1* expression levels in resistant isolates when compared to susceptible isolates. While there was no statistically significant difference in the *CDR2*, *PDR1* expression levels as shown in table (6). On the other hand, *C. auris*, the expression level for fluconazole resistance genes *(ERG11*, *CDR1*, *CDR2* and *MDR1)* showed statistically significant difference in all tested genes in resistant isolates when compared to susceptible isolates (table 7).

Table (3): Demographic data of patients with positive candidiasis

Item		n= 51 no (%)
Sex	Male	18 (35.3%)
	Female	33 (64.7%)
Age (years)	Mean ±SD	43.3 ± 17.1
	Range	1 – 67
Risk factors	Diabetes	10 (19.6%)
	Chronic diseases	7 (13.7%)
	Napkin dermatitis	5 (9.9%)
	Vaginitis	16 (31.4%)
	COVID 19	5 (9.9%)
	ICU, antibiotics	8 (15.7%)

Table (4): Different methods used in speciation of *Candida* isolates

Candida isolates	Germ tube test	Growth at 45°C	Hicrome Candida differential agar	VITEK 2 compact system
C. albicans	Positive	Positive	Green	Excellent
C. tropicalis	Negative	Negative	Blue	Excellent
C. auris	Negative	Positive	White tinged with mauve	Very Good
C. krusie	Negative	Negative	Pink	Excellent identified
C. glabrata	Negative	Negative	White	Excellent identified
C. famata	Negative	Negative	White	Good
C. gluilliermondii	Negative	Negative	White	Good
C. dubliniensis	Positive	Negative	Green	Good

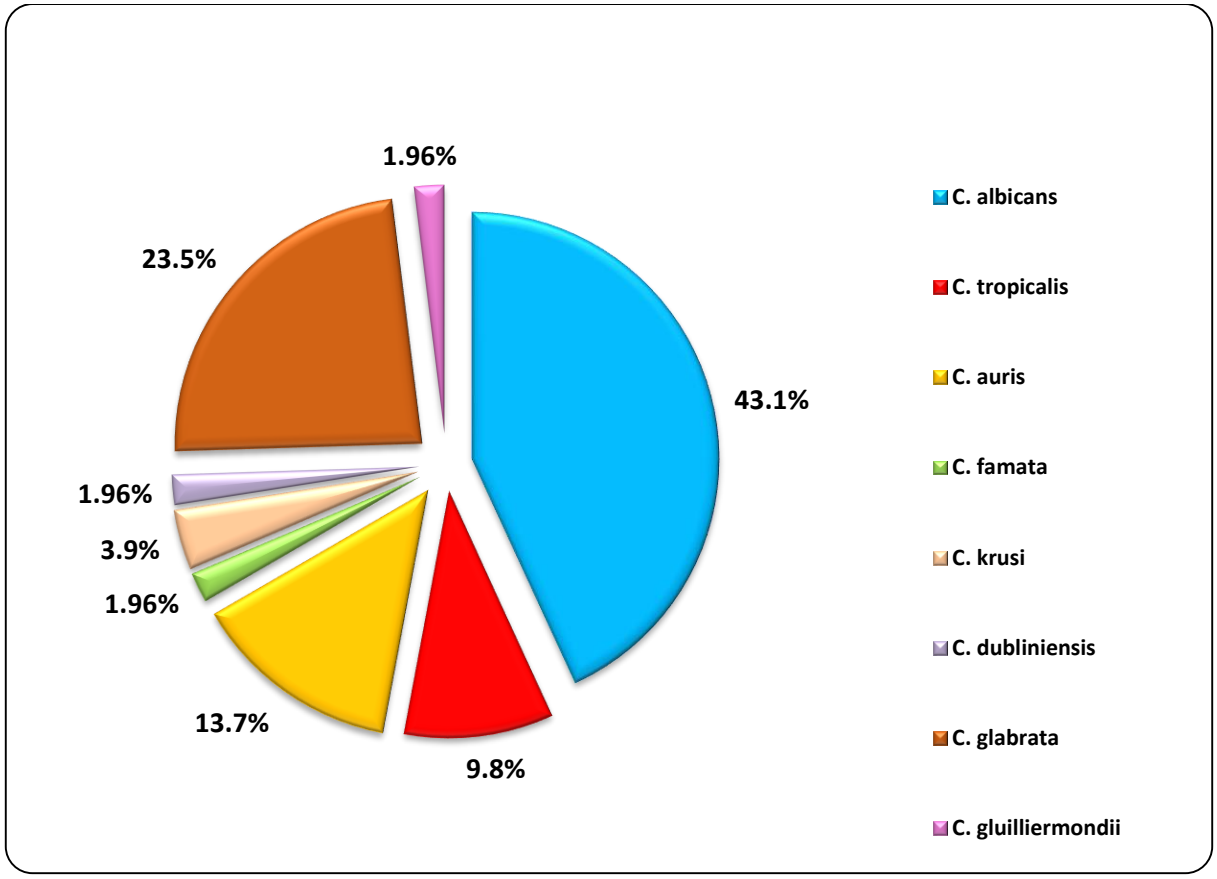

Figure (1): Distribution of *Candida* species among *Candida* isolates

Table (5): Distribution of fluconazole resistance in *Candida spp.*

Species	n= 20 no. (%)
C. glabrata	8 (40%)
C. auris	7 (35%)
C. krusie	2 (10%)
C. famata	1 (5%)
C. dubliniensis	1 (5%)
C. guilliermondii	1 (5%)

***C. krusie* exhibit intrinsic resistance**

Table (6): Mean relative gene expression levels in *C. glabrata* isolates

Gene	Mean relative gene expression levels		Stat. test	p-value
	Resistant Mean ± SD	Sensitive Mean ± SD		
ERG11	4.91 ± 3.25	1.04 ± 0.31	t= 3.3	0.012*
CDR1	2.10 ± 1.23	1.0 ± 0.01	t= 2.5	0.04*
CDR2	1.18 ± 0.34	1.0 ± 0.08	t= 1.03	0.327
PDR1	1.42 ± 0.54	1.21 ± 0.79	t= 0.55	0.591

t: independent sample T test, *: Significant p-value (< 0.05)

Table (7): Mean relative gene expression levels in *C. auris*

Gene	Mean relative gene expression levels		Stat. test	p-value
	Resistant Mean ± SD	Sensitive Mean ± SD		
CDR1	5.37 ± 1.74	1.54 ± 0.71	t= 4.1	0.003*
CDR2	12.4 ± 6.02	1.42 ± 0.13	t= 3.5	0.006*
ERG11	7.97 ± 3.21	1.01 ± 0.15	t= 4.2	0.002*
MDR1	4.44 ± 2.89	1.01 ± 0.2	t= 2.3	0.046*

t: independent sample T test, *: Significant p-value (< 0.05)

DISCUSSION

In hospital environments, *Candida* species are becoming more and more prevalent as the primary pathogens of opportunistic illnesses. Thus, to minimize morbidity and death, early isolation, speciation, and antifungal susceptibility testing are crucial for doctors in selecting the optimal therapeutic route for patient [3].

In the present study, the prevalence of candidiasis was (17%) of all participated patients. A similar result was found in Mansoura University hospitals in Egypt by Elnagar et al. [1] and in India by Rengaraj and Bharathidasan [9]. In contrast, other study in Egypt was conducted by Mohamed et al. [10] who reported a much higher prevalence of candidiasis that accounted 55%. This variation could be attributed to the difference in patients' numbers from each district, socioeconomic status of sample patients, poor lifestyle choices concerning self-care and personal hygiene. The results of this investigation, which were consistent with those published by Chongtham et al. [3] and Mohamed et al. [10], who showed that females had a greater preference for *Candida* species. Conversely, the findings contradict those of Zeng et al. [11], who found that males had a higher frequency of invasive *Candida* infections. Males and females of the *Candida* species were found to be almost equally distributed by Marak and Dhanashree [12]. This difference may be because most of samples in the present study were vaginal swab from females. Moreover, most of isolates were from geriatric age group (> 60 years) which was comparable to research published by Chongtham et al. [3] and Mohamed et al. [10]. This population is more prone to have comorbid conditions leading to immunosuppression and *Candida* infection.

Concerning the present study, prevalence of *Candida* was associated with multiple risk factors; vaginitis was the most common predisposing risk factor followed by diabetes, ICU stay, antibiotic use, chronic diseases, covid 19 infection and napkin dermatitis. This was similar with findings conducted by Ghimire et al. [2] and Chongtham et al. [3].

Concerning the identification of *Candida* in the present study by conventional methods using germ tube test and growth at 45°C, most of the *Candida* strains couldn't be differentiated by these methods. As, germ tube test was found to be positive in *C. albicans* and *C. dubliniensis* only as reported by Ahmad et al. [13] and only *C. albicans* and *C. auris* can grow at 45°C as reported by Borah and Sharma [6].

The present study revealed that both of *C. albican* and *C. dubliniensis* produce green colonies, *C. tropicalis* produce blue colonies, *C. krusie* produce pink colonies as reported by Chongtham et al. [3], *C. auris* produce white tinged with mauve colonies as reported by Borah and Sharma [6] while all other *non albicans spp.* produce white colonies on Hicrome Candida Differential agar. In the current work, Vitek-2 Compact automated system was quite consistent in identification of both *C. albicans* and *NAC spp.* including *C. tropicalis*, *C. auris*, *C. krusi*, *C. glabrata*, *C. famata*, *C. gluilliermondii* and *C. dubliniensis* with acceptable discrimination power. A preceding study in Egypt agreed with these findings was conducted by Elnagar et al. [1].

In the present study, it was noticed that *NAC* were more prevalent (56.9%) than *C. albicans* (43.1%). Out of 29 (56.9%) *NAC spp*., there were 12 (23.5%) *C. glabrata*, 7 (13.7%) *C. auris*, 5 (9.8%) *C. tropicalis*, 2 (3.9%) *C. krusi*,1 (1.96%) *C. famata*, 1 (1.96%) *C. dubliniensis* and 1 (1.96%) *C. gluilliermondii* among the isolated strains. These findings in agreement with a study conducted by Ghazi et al. [14] in the Middle East and

North Africa**,** Warghade et al. [15] in India and Abdel-Hamid et al. [16] in Brazil. In contrast to these findings, there were studies showed that *C. albicans* was more prevalent than *NAC* in Egypt by Elnagar et al. [1] and Mohamed et al. [10], in India by Rengaraj and Bharathidasan [9] and Ghimire et al. [2] in Nepal. In the current study, *C. auris* was the second predominant isolated spp., isolated during second wave of covid 19 suggested complications and high mortality as it couldn't be diagnosed. This corresponds with a study that was carried out in Qatar by Abid et al. [17] who reported that most documented cases of C. auris in 2020-2021 were in patients with COVID-19.

In the present study, Vitek-2 Compact system couldn't detect susceptibility pattern of all antifungal drugs in *C. auris*, and susceptibility to fluconazole only in *C. glabrata*. Several studies support these findings about limitation of Vitek-2 system to detect antifungal susceptibility pattern of different *Candida spp.* reported by Mohamed et al. [10] and Rengaraj and Bharathidasan [9]. This may be because the Vitek-2 system may require a longer incubation period to calculate the MIC endpoints of the medications against these isolates, or it may be because the MIC values of the drugs against these isolates are not listed in the system database. While disk diffusion method could detect fluconazole susceptibility pattern for *C. auris* and *C. glabrata*, it was found that it could overcome limitation of Vitek-2 system. Nunnally et al. [18] support these findings about effectiveness of this method in *C. auris* and Jeon et al. [19] in *C. glabrata*.

Regarding the antifungal susceptibility obtained by Vitek 2 compact automated system and disc diffusion method for fluconazole; out of 51 isolates, 20 (39.2%) were resistant to fluconazole, distributed between *C. glabrata, C. auris, C. krusie, C. famata, C. guilliermondii, C. dubliniensis*, similar results conducted by Mohamed et al. [10] and Rengaraj and Bharathidasan [9]. The primary cause of high degree of resistance to fluconazole is the abuse of this antifungal drug and empirical therapy because of its broad-spectrum activity against different species of *Candida*, low cost, and strong efficacy.

In the present study, concerning analysis of *C. glabrata* gene expression there was statistically significant difference in the *ERG11* expression levels in resistant isolates compared to sensitive isolates, which agrees with Mohamed et al. [10] and Fathi [20]. In contrast with these results, Hatami et al. [5], Abd-Alrahman et al. [4], Zhang et al. [21] and Whaley et al. [22] found that no statistically significant changes in transcription levels between the clinical isolates that are sensitive to fluconazole and those that are resistant to it in *C. glabrata*. As regards *CDR1* expression level in the current work, it was statistically significant in resistant isolates relative to susceptible isolates. This agreed with several studies performed by Hatami et al. [5], Abd-Alrahman et al. [4], Yao et al. [23] and Whaley et al. [22].

In this study, there was no statistically significant difference in the *CDR2* and *PDR1* expression levels between resistant and sensitive isolates. Similar to study reported by Hatami et al. [5] about *CDR2* and Yao et al. [23] about *PDR1***.** In contrast, Hatami et al. **[5],** Abd-Alrahman et al. [4] and Cavalheiro et al. [24] who ensured in their studies about the role of *PDR1* in fluconazole resistance isolates.

In the current study, concerning *C. auris* efflux pump gene expression (*CDR1, CDR2, MDR1*) and *ERG11* analysis which was performed using RT-qPCR and calculated by ΔΔCT (cycle threshold) method, there was statistically significant difference in resistant isolates relative to susceptible isolates in all tested genes. Our results agreed with Wasi et al. [25], Abid et al. [17] in Qatar and Chowdhary et al. [26].

CONCLUSION

There was an increase in *non albicans Candida* infection and emergence of fluconazole resistant species. Immunological changes caused by COVID- 19 and patient-related risk factors have been implicated in the pathogenesis of *Candida* co-infection and emerging of multidrug-resistant *C. auris*. Most of the clinical resistance in *C. glabrata, C. auris* could be attributed to the upregulation of ABC transporters. *C. auris* exhibited a higher significant efflux pump activity than *C. glabrata*. Based on this work it is predicted that they play an essential role in azole resistance.

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الملخص العربي

جهازالفيتك٢ في تعريف واختبارالحساسية لفصائل الكانديدا والكشف عن الجينات المقاومه لعقارالفلوكونازول

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ملخص البحث:

الخلفية : تعد الزيادة في معدلات الاصابة بالعدوي الفطرية وظهور سلالات جديدة مسببة لعدوي المستشفيات من الاسباب الضرورية لدراسة فطر الكانديدا ، وحيث ان هناك العديد من الآليات التى توضح طرق مقاومة الكانديدا لعقار الفلوكونازول منها طريقة طرد العقار خارج الخلية بواسطة مضخة التدفق وكذلك التعبير الجيني المتزايد لجين *ERG 11* فى السلالات المقاومة.

الهدف: تحديد معدل انتشار فصائل الكانديدا المعزولة من العينات الإكلينيكية المختلفة والتعريف السريع بها وإختبار مدى حساسية مضادات الفطريات مع الكشف عن مستويات التعبير الجيني للسلالات المقاومة لعقار الفلوكونازول.

الطرق: بعد الحصول علي موافقة المرضى تم جمع ثلاثمائة عينة إكلينيكية مختلفة من المرضى المحجوزين بالاقسام المختلفة بمستشفى طنطا الجامعى ، وتم التعرف علي الكانديدا بالطرق التقليدية والتأكد من تلك الفصائل عن طريق جهاز الفيتك ٢ ، كما تم عمل إختبار الحساسية لعقارالفلوكونازول وغيره من أدوية مضادات الفطريات بواسطة جهاز الفيتك٢ و طريقة الانتشار القرصي ، وتم استخدام اختبار تفاعل البلمرة المتسلسل الكمي لتقييم مستويات التعبير الجيني لمقاومة *الفلوكونازول ERG11* ، *CDR1* ، *CDR2* و *PDR1* في عزلات المبيضات الجرداء وكذلك *ERG11* ، *CDR1* ، *CDR2* و*MDR1* في عزلات مبيضات الاوريس حيث كونها الفصائل المقاومة الاكثر شيوعا في هذا البحث.

النتائج: تم فصل 51 سلاله من المبيضات من 300 عينه إكلينيكية مختلفة بنسبة (17%)، حيث كانت نسبة المبيضات الغير البيكانز (56.9%) اعلي من المبيضات الالبيكانز(43.1%) وقد أظهرت الدراسه ان نسبة المبيضات المقاومه لعقار الفلوكونازول 20 من 51 من السلالات المعزوله بنسبة (39.2%) وقد أظهرت تجارب تفاعل البلمرة المتسلسل الكمي في عزلات المبيضات الجرداء أن هناك فرق ذو دلالة إحصائية في مستويات التعبيرلجينات*EGR11* و*CDR1* بين العزلات المقاومة والعزلات الحساسة بينما لم يكن هناك فرق معتد به إحصائياً في مستويات التعبيرلجينات *CDR2* و *PDR1* في العزلات المقاومة للفلوكونازول مقارنة بالعزلات الحساسة ، بينما أظهر التعبير الجيني في مبيضات الاوريس ان هناك فرق ذو دلالة إحصائية في مستويات التعبيرلجميع الجينات.

الأستنتاجات: تأكيد ارتفاع معدل الاصابة بعدوى الكانديدا الغيرالبيكانز*non albicans Candida* فى المرضي مع انتشار العزلات المقاومة لعقار الفلوكونازل، وقد تم عزل مبيضات الاوريس المقاومة اثناء تفشي وباء كوفيد ١٩ كما أظهر التعبير الجيني في مبيضات الاوريس.

الكلمات المفتاحيه: جهاز الفيتك2، المبيضات الغير البيكانز، العزلات المقاومة للفلوكونازول، تفاعل البلمرة المتسلسل الكمي، مبيضات الاوريس.

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