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Research Article

Evaluation of Susceptibility of *Candida* species to Six Antifungal Drugs in Iraqi Specimens

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A B S T R A C T

Introduction: Candida spp. has become increasingly resistant to antifungal drugs, with elevated MIC levels causing a negative medical impact and increasing the number of patients at risk of candidiasis. According to the CDC, about 7% of *Candida* blood samples show reduced susceptibility to fluconazole. Monitoring the antifungal resistance profile of *Candida* spp. is vital, as non-Albicans species may limit treatment options.

Objective: Evaluate the antifungal effectiveness against clinical *Candida* spp. isolates of six antifungals: amphotericin B, fluconazole, voriconazole, itraconazole, caspofungin, and 5-fluorocytosine.

Methods: 100 samples were collected from various clinical samples at the National Centre of Teaching Laboratories in Baghdad, Iraq, from May to December 2023. The effectiveness of six antifungals (fluconazole (FLC), itraconazole (ITR), voriconazole (VRC), amphotericin B (AMB), caspofungin (CAS), and 5-fluorocytosine (5-FC)) was tested using the MA120 Automated ID and AST System (Render) according to CLSI standards.

Results: Out of 100 isolates, nine *Candida* species were identified: C. *albicans* (54%), C. *glabrata* (20%), C. *dubliniensis* (10%), C. *tropicalis* (6%), C. *krusei* (5%), C. *parapsilosis* (2%), and C. r*ugosa*, C. *lusitaniae*, and C. *kyfer* (each 1%). The non-susceptible rates to the six antifungals were: 5-FC (42%), FLC (21% intermediate, 9% resistant), AMB (11%), ITR (8%), VRC (6%), CAS (4% intermediate, 1% resistant).

Conclusion: We observed increased resistance rates to 5-FC, FLC, ITR, AMB, and VRC, but not to caspofungin. C. *albicans* showed a high 5-FC non-WT phenotype (72%) with elevated MIC values, while C. *glabrata* had a 7% non-WT rate against AMB. C. *tropicalis* and C. *parapsilosis* revealed limited susceptibility to azoles.

Keywords: Antifungals, *Candida*, Susceptibility, Resistance, Diseases

Introduction

The most prevalent fungi that cause fungaemia are of the genus *Candida*. Depending on the location of the infection and the patient's immunity, *Candida* infections can range in severity from mild to lethal. *Candida* causes a range of invasive life-threatening illnesses, from bloodstream infection to non-critical mucocutaneous candidiasis like vulvovaginal, genitourinary, and oropharyngeal. Invasive candidiasis is frequently linked to a high risk of death rate (1.5 million deaths annually); invasive candidiasis increases the length of hospitality.[1] C. *albicans* is considered a prevalent species causing candidiasis. Although, lately, an epidemiological change for non-albicans *Candida* spp. infections occurred, which exhibited elevated MIC values for azoles and echinocandin.[2,3]

The selection of a particular antifungal depends on the patient's clinical condition, the drug's relative toxicity and efficacy in the target patient group, the species of the infecting isolate, susceptibility to antifungals, and the patient's pre-exposure to drug medications.[4] Unfortunately, treatment options for candidiasis are limited. Despite the availability of many types of antifungals, only a few main divisions are used for human treatment. Based on their action mechanisms, the antifungal medications that are currently used to treat candidiasis are categorised into four classes: (1) Alteration of cell membrane sterol (polyenes, including amphotericin B, Nystatin); (2) Ergosterol biosynthesis pathway inhibition (azoles, including fluconazole, voriconazole, posaconazole, and ravuconazole); (3) inhibition of DNA or RNA synthesis (flucytosine) (4) Glucan synthesis inhibition (caspofungin, micafungin, and anidulafungin which are echinocandins).[5]

Several combined factors have contributed to the clinical failure of candidiasis treatment in recent years; among them are fungal pathogen factors, patient factors, drug kinetics and dynamics, and medication spread at the infected location. These factors all play a part in therapeutic effectiveness and resistance development.[6] The rising incidence of antifungal resistance significantly complicates patient management and has negative economic effects since there are only a few classes of antifungal medications and no vaccines invented against *Candida*. The increased candidiasis incidence leads to increased drug usage, and the application of subtherapeutic dosages of fungistatic antifungals, both general and long-term, leads to the acquisition of resistance against drugs.[7]

The high safety profile of Azoles leads to their worldwide usage to treat *Candida*, as they are cheap, show reduced toxicity, and are available for oral usage. One of the biggest handicaps to achieving clinical success with azole is the resistance among species of *Candida* and *Aspergillus*.[8,9] Due to prolonged azole usage worldwide, *Candida* species resistant to azoles have increased, as the MICs level is rising and failure of clinical treatment.[10] *Candida* species examined worldwide, > 2.5% and > 9% of the yeast show resistance to both fluconazole and itraconazole.[11]

Despite that azole and echinocandins are pioneers in treating candidiasis, *Candida* species have progressed resistance against them.[12] The echinocandin drugs are used for yeasts that show resistance to azoles, especially C. krusei and C. *glabrata*. Prolonged exposure to echinocandin reduced *Candida*'s susceptibility to the drug. Higher MIC levels with failure of occasional treatment have been confirmed for strains of *Candida*, especially C. *glabrata*.[13] Despite the growing resistance against the echinocandin class, the rate of echinocandin resistance among *Candida* species is still relatively low (1–3%).[14]

Polyenes, such as amphotericin B, are the antifungal drug with a wide range spectrum, used for systemic fungal infections. Although amphotericin B has been utilized for more than 70 years, resistance to this drug is still rare.[15] However, there is significant host toxicity that limits its usage.[16] The absence of distinct susceptibility breakpoints is a significant obstacle to the study of polyene resistance epidemiology. Some studies showed that the frequently employed breakpoint for *Candida* species is 2 µg/mL.[17] The majority of isolates that are resistant to antifungals are uncommon species of *Candida*, including C. *glabrata*, C. *guilliermondii*, and C. *lusitaniae*.[18]

Flucytosine affects many *Candida* species isolates *in vitro*. It is usually used along with other antifungal drugs like AmB or triazoles, for treating yeast infections that are invasive due to their high toxicity. Important flucytosine usage is often limited due to the quick emergence of drug resistance among *Candida* species during treatment. Nearly 10% of *Candida* species intrinsically show resistance to flucytosine.[19–21]

In the current study, isolates of Candida were tested against six antifungal drugs, which are fluconazole, itraconazole, voriconazole, amphotericin B, caspofungin, and 5-fluorocytosine. Depending on the results, the most effective antifungal drug against candidiasis in Iraq was determined. Also, the highly resistant species of Candida against the studied drugs were revealed.

Materials and Methods

Sampling

This cross-sectional study was performed on a total of 100 samples collected during the period from May 2023 to December 2023. Different clinical samples were collected from candidiasis patients (44 male and 56 female). Clinical samples included sputum, bronch-alveolar lavage, vaginal swab, urine, mouth swab, blood, and CSF. Different clinical samples were cultured from patients with candidiasis.

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The Ethics Committee at the Department of Biology in Mustansiriyah University and the Iraqi Ministry of Health accepted the study proposal and protocol (reference: CSEC/0512/0065).

Antifungal Susceptibility Test

All isolates were submitted for identification and susceptibility testing against six antifungal drugs, including flucytosine, amphotericin, caspofungin, fluconazole, itraconazole, and voriconazole, using the ID&AST System MA120 (Render, China). The susceptibility test was conducted according to CLSI standards.

Reading Susceptibility Test Results

The MIC breakpoint or epidemiological cutoff value (ECV) values for Candida species of the CLSI M59 and M60 guideline standards were adopted to determine the susceptibility pattern; some drugs have no CLSI breakpoint or ECV, therefore we applied the values that were determined by previous studies in the field.

Statistical Analysis

The study employed the Statistical Analysis System (SAS) (2018) programme to determine the impact of various variables on the study parameters. In this study, a chi-square test was employed to compare the percentage (0.05 and 0.01) probability in a meaningful way.

Results

Different clinical samples were included in the current study; the major number of Candida isolates were obtained from respiratory specimens (sputum, bronch-alveolar lavage) 76 (76%) with high significant differences ($p < 0.01$), followed by vaginal swab 9 (9%), urine 7 (7%), mouth swab 6 (6%), and blood and CSF, which each represented 1 (1%) of specimens (Table 1). The isolate was obtained from 100 patients; 56 (56%) specimens were from female patients, while 44 (44%) specimens were from male patients. Males and females demonstrated no significant differences (Table 1).

Table 1.Divisions of *Candida* spp. in Different Clinical Specimens Including Sex Groups

Site of Sample	n (%)	Male	Female
Respiratory specimen	76 (76.00)	39	37
Vagina	9 (9.00)	---	9
Urine	7 (7.00)	3	4
Mouth	6 (6.00)	2	4
Others (CSF, blood)	2 (2.00)	-	2
Total	100 (100.00)	44 (44.00)	56 (56.00)
p value	197.30** (0.0001)	1.440 (0.230)	

**p ≤ 0.01; highly significant differences

Non-significant differences between sex groups

The present study results showed that most candidiasis patients are of the age group 40–60, followed by older patients (more than 60 years of age), followed by younger patients belonging to the age group of 20–40 years, and at the end, younger patients of the age group of less than 20 years. There was a high significant difference ($p < 0.01$) in the infection rate between patients of different age groups (Table 2).

Table 2.Samples Distribution According to Age Groups

Age Groups (Years)	n (%)
< 20	10 (10)
20–40	22 (22)
40–60	36 (36)
> 60	32 (32)
Total	100 (100)
Chi-square (p value)	16.160** (0.0010)

**p ≤ 0.01

Incidence of Candida species

A total of 100 samples were obtained from *Candida*-infected patients. Nine different species were identified, including C. *albicans*, C. *glabrata*, and C. *dubliniensis*. C. *tropicalis*, C. *krusei*, C. *parasilosis*, C. *rugosa*, C. *lusitaniae*, and C. *kyfer*). The epidemiology of C. *albicans* was 54%, followed by C. *glabrata* (20%), C. *dubliniensis* (10%), C. *tropicalis* (6%), C. *krusei* (5%), C. *parapsilosis* (2%), and the last three species, C. *rugosa*, C. *lusitaniae*, and C. *kefyr*, each representing 1% of isolates. A highly significant difference was revealed by statistical analysis ($p < 0.01$) among Candida species epidemiology (Table 3).

Table 3.Divisions of *Candida* Species

Candida Species	Number of Isolates (%)
C. albicans	54 (54.00)
C. glabrata	20 (20.00)
C. dubliniensis	10 (10.00)
C. tropicalis	6 (6.00)
C. krusei	5 (5.00)

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C. parasilosis	2 (2.00)
Other *Candida species* (C. *rugosa*, C. *lusitaniae*, C. *kyfer*)	3 (3.00)
Total	100 (100.00)
Chi-square (p value)	37.026** (0.0001)

**p ≤ 0.01

Antifungal Susceptibility Profiling Results

This study tested *Candida isolates* against six antifungals: fluconazole (FLC), voriconazole (VRC), and itraconazole (ITR), and the other three were amphotericin B (AMB), caspofungin (CAS), and flucytosine (5-FC). Overall results showed that *Candida* is highly susceptible to all tested antifungals with high significant differences ($p < 0.01$). 70 (70%) isolates were susceptible to FLC, 21 (21%) were intermediate, and 9 (9%) were resistant, while for ITR, 92 (92%) isolates showed a wild-type (WT) phenotype and only 8 (8%) isolates had a non-wild-type (non-WT) phenotype. Dealing with VRC, 93 (93%) isolates were susceptible, only 1 (1%) isolate was intermediate, and 6 (6%) were resistant. For AMB, 89 (89%) isolates showed a WT phenotype and 11 (11%) isolates showed a non-WT phenotype. For CAS, 95 isolates were susceptible, 4 (4%) were intermediate, and 1 (1%) isolate was resistant. For 5-FC, 58 (58%) isolates showed a WT phenotype and 42 (42%) isolates showed a non-WT phenotype (Table 4).

Table 4.Susceptibility of Candida Species Against the Six Studied Antifungals

		Antifungal Drugs (n, %)					
Species	**Pattern**	**FLC**	**ITR**	**VRC**	**AMB**	**CAS**	**5-FC**
C. *albicans*	S/WT I R/NWT	50 (92.6) - 4 (7.4)	50 (92.6) - 4 (7.4)	53 (98.1) 1 (1.9) -	53 (98.1) - 1 (1.9)	54 (100.0) - -	15 (27.8) - 39 (72.2)
C. *glabrata*	S/WT I R/NWT	- 20 (100.0) -	20 (100.0) - -	20 (100.0) - -	13 (65.0) - 7 (35.0)	16 (80.0) 4 (20.0) -	20 (100.0) - -
C. *dubliniensis*	S/WT I R/NWT	9 (90.0) - 1 (10.0)	8 (80.0) - 2 (20.0)	9 (90.0) - 1 (10.0)	8 (80.0) - 2 (20.0)	10 (100.0) - -	9 (90.0) - 1 (10.0)
C. *tropicalis*	S/WT I R/NWT	3 (50.0) 1 (16.7) 2 (33.3)	6 (100.0) - -	3 (50.0) - 3 (50.0)	6 (100.0) - -	6 (100.0) - -	6 (100.0) - -
C. *krusei*	S/WT I R/NWT	5 (100.0) - -	5 (100.0) - -	5 (100.0) - -	4 (80.0) - 1 (20.0)	5 (100.0) - -	5 (100.0) - -
C. *parapsilosis*	S/WT I R/NWT	- - 2 (100.0)	- - 2 (100.0)	2 (100.0) - -	2 (100.0) - -	2 (100.0) - -	2 (100.0) - -
Others	S/WT I R/NWT	3 (100.0) - -	3 (100.0) - -	1 (33.3) - 2 (66.7)	3 (100.0) - -	2 (66.7) - 1 (33.3)	1 (33.3) - 2 (66.7)
All isolates	S/WT I R/NWT	70 (70.0) 21 (21.0) 9 (9.0)	92 (92.0) - 8 (8.0)	93 (93.0) 1 (1.0) 6 (6.0)	89 (89.0) - 11 (11.0)	95 (95.0) 4 (4.0) 1 (1.0)	58 (58.0) - 42 (42.0)

FLC: Fluconazole, AMB: Amphotericin B, VRC: Voriconazole, CAS: Caspofungin, 5-FC: Fluorocytosine, ITR: Itraconazole, R: Resistant, I: Intermediate, WT: Wild type, NWT: Non-wild type

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Candida albicans Susceptibility Profiling

Albicans spp. was highly susceptible to FLC (MIC ≤ 2–4 µg/mL), 50 isolates were susceptible, and 4 isolates showed resistance against FLC (MIC ≥ 8 µg/mL). For ITR, 50 isolates showed a WT phenotype, while only four samples revealed a non-WT phenotype (ECV 0.12 µg/mL). For VOR, 53 isolates were susceptible (MIC ≤ 0.12 µg/mL), only one isolate showed intermediate (MIC 0.25–0.5 µg/mL), and no resistance was demonstrated against VOR. It showed a WT phenotype against AMB 53, while only one isolate showed a non-WT phenotype (ECV 2 µg/mL). For CAS, C. albicans showed no resistance against the drug; all 54 samples were susceptible (MIC ≤ 0.25 µg/mL). There is neither a clinical breakpoint (CBP) nor epidemiological cut-off value (ECV) determined by CLSI for 5-FC. The ECV (0.5 µg/mL) was applied according to Pfaller et al.'s study.[22] Fifteen isolates showed a WT phenotype, while 39 samples demonstrated a non-WT phenotype (ECV 0.5 µg/mL) (Table 4).

Candida glabrata Susceptibility Profiling

For FCL, all 20 isolates were S-DD (MIC ≤ 32 µg/mL). For ITR, all samples revealed the Wild type phenotype (ECV 2 µg/mL). For VRC, all 20 isolates showed the WT phenotype (ECV 0.25 µg/mL). For AMB, 13 isolates showed a WT phenotype and seven demonstrated a non-WT phenotype (ECV 2 µg/mL). CAS C. *glabrata* showed no resistance against the drug, as 16 isolates were susceptible (MIC ≤ 0.12 µg/mL), and four samples were intermediate (MIC 0.25 µg/mL). For 5-FC, all samples presented the WT phenotype (ECV 0.5 µg/mL).

Candida dubliniensis Susceptibility Profiling

For FLC, 9 isolates showed the WT phenotype, and only 1 isolate showed the non-Wild type phenotype (ECV 0.5 µg/mL). For ITR, eight samples presented a WT phenotype, while two samples presented a non-WT phenotype (ECV 0.25 µg/mL). For VRC, 9 isolates showed the WT phenotype and only 1 isolate showed non-WT (ECV > 0.06 µg/mL).[23] For AMB, 8 isolates were detected as WT and 2 were detected as non-WT (ECV 0.5 µg/mL). For CAS, all 10 isolates showed the WT phenotype (ECV ≤ 0.12 µg/mL).[24] For 5-FC, nine isolates showed the WT phenotype and only one showed the inverse (Table 4).

C. tropicalis Susceptibility Profiling

For FLC, three isolates were susceptible, only one isolate was S-DD, and two isolates were resistant. For ITR, all six isolates showed the WT phenotype. For VRC, three samples were susceptible (MIC ≤ 0.12 µg/mL), and three samples were resistant. For AMB, six samples presented the WT phenotype (ECV 2 µg/mL). For CAS, all samples were susceptible. In 5-FC, all six samples revealed a WT phenotype (ECV 0.5 µg/mL) (Table 4).

C. krusei Susceptibility Profiling

For FLC, all isolates showed the WT phenotype. An ECV of ≥ 64 µg/mL was applied according to Pfaller and Diekema because C. krusei samples were intrinsically resistant to fluconazole (according to CLSI). For ITR, all samples presented the WT phenotype. For VRC, all samples were susceptible. For AMB, 4 isolates showed a WT phenotype, while only 1 isolate showed a non-WT phenotype (ECV 2 µg/mL). For CAS, all samples were susceptible. Dealing with 5-FC, the ECV of Pfaller et al. also used ECV 32 µg/mL, and all isolates showed the WT phenotype (Table 4).[22]

Less Common *Candida* spp. Susceptibility Profiling

C. *lusitaniae* was the only isolate included in this study; this isolate showed a WT phenotype for FLC (ECV 1 µg/mL), ITR (ECV 1 µg/mL), VOR (ECV 0.03 µg/mL), AMB (ECV 2 µg/mL), CAS (ECV 1 µg/mL), and 5-FC (ECV 0.5 µg/mL). Only two cultures of C. *parapsilosis* were included in the current study. For FLC, both cultures were resistant (MIC ≥ 8 µg/mL); for ITR, both cultures revealed a non-WT phenotype (ECV 0.5 µg/mL). For VOR, both sample cultures were susceptible to the drug (MIC ≤ 0.12 µg/mL). Both sample cultures showed the WT phenotype (ECV 1 µg/mL) for AMB. For CAS, both sample cultures were susceptible to the drug (MIC ≤ 2 µg/mL). For 5-FC, both isolates showed the WT phenotype (ECV 0.5 µg/mL). C. *kefyr* was the only isolate included in the current study. C. *kefyr* showed a WT phenotype for FLC (ECV 1 µg/mL), ITR (ECV 0.5 µg/mL), AMB (ECV 2 µg/mL), and 5-FC (ECV 0.5 µg/mL). The isolate showed a non-WT phenotype for VRC (ECV 0.015 µg/mL) (28) and CAS (ECV 0.03 µg/mL).[23] According to MIC, the isolate was susceptible to FLC (8 µg/mL), ITR (2 µg/mL), CAS (1 µg/mL), AMB (2 µg/mL), and resistant to VOR (0.12 µg/mL) and 5-FC (2 µg/mL) (Table 5).

Table 5.Antifungal Susceptibility Profile of 100 Candida Isolates

	No. of Isolates at Each Determined MIC Value (µg/mL)																
	Drug	≤ 0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	> 128	MIC Range (µg/mL)	GM MIC (µg/L)

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C. albicans	FLC	-	-	-	-	14	7	1	28	-	1	2	-	-	1	0.2–> 128	3.28
	ITR	-	2	44	4	-	2	-	-	-	2	-	-	-	-	0.03–8	0.24
	VRC	-	13	2	38	1	-	-	-	-	-	-	-	-	-	0.03–0.25	0.85
	AMB	-	-	-	4	23	17	9	-	-	-	1	-	-	-	0.12–16	0.75
	CAS	-	-	-	48	6	-	-	-	-	-	-	-	-	-	0.12–0.25	0.17
	5-FC	-	-	15	-	-	-	7	28	1	-	1	1	1	-	0.06–64	3.97
C. glabrata	FLC	-	-	-	-	-	-	1	11	3	3	2				1–16	5.00
	ITR	-	-	7	1	3	5	3	1	-	-	-				0.06–2	0.34
	VRC	-	-	-	15	5	-	-	-	-	-	-				0.12–0.25	0.17
	AMB	-	-	-	-	6	3	2	2	6	1	-				0.25–8	1.41
	CAS	-	-	-	16	4	-	-	-	-	-	-				0.12–0.25	0.17
	5-FC	-	-	-	-	-	-	2	11	3	3	-	1				4.59
C. dubliniensis	FLC	-	-	-	-	7	2	-	1	-	-	-				0.25–2	0.62
	ITR	2	-	4	2	-	-	-	-	-	2	-				≤ 0.015–8	0.17
	VRC	-	9	-	-	-	-	1	-	-	-	-				0.03–1	0.17
	AMB	-	-	-	-	8	-	-	1	1	-	-				0.25–4	1.25
	CAS	-	-	-	10	-	-	-	-	-	-	-				0.12	0.12
	5-FC	-	-	8	-	-	1	1	-	-	-	-				0.06–1	0.31
C. tropicalis	FLC	-	-	-	-	-	-	1	2	1	-	-		2		1–64	4.75
	ITR	-	-	4	2	-	-	-	-	-	-	-				0.06–0.12	0.08
	VRC	-	-	-	3	-	-	-	-	3	-	-				0.12–4	0.69
	AMB	-	-	-	-	1	-	2	1	2	-	-				0.25–4	1.18
	CAS	-	-	-	6	-	-	-	-	-	-	-				0.12	0.12
	5-FC	-	-	-	-	-	-	1	5	-	-	-				1–2	1.41
C. krusei	FLC	-	-	-	-	-	-	-	-	-	3	1	1			8–32	16.00
	ITR	-	-	-	1	-	4	-	-	-	-	-				0.12–0.5	0.24
	VRC	-	-	-	2	2	1	-	-	-	-	-				0.12–0.5	0.24
	AMB	-	-	-	-	-	-	2	2	1	-	-				1–4	2.00
	CAS	-	-	-	4	1	-	-	-	-	-	-				0.12–0.25	0.17
	5-FC	-	-	-	-	-	-		-	1	2	2					8.00

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Others	FLC	-	-	-	-	-	1	1	1	-	-	-		1	1	0.5–>128	9.51
	ITR	-	-	1	1	1	-	-	-	-	2	-				0.06–8	0.34
	VRC	-	1	-	3	1	-	-	-	-	-	-				0.03–0.25	0.09
	AMB	-	-	-	1	2	2	-	-	-	-	-				0.12–0.5	0.24
	CAS	-	-	-	3	2	-	-	-	-	-	-				0.12–0.25	0.17
	5-FC	-	-	1	1	-	-	2	1	-	-	-				0.06–2	0.34

FLC: Fluconazole, ITR: Itraconazole, VRC: Voriconazole, AMB: Amphotericin B, CAS: Caspofungin, 5-FC: Fluorocytosine, MIC: Minimum Inhibitory Concentration, GM: Geometric mean

Discussion

Epidemiologically, our results show diversity in *Candida* species. Nine types were isolated from examined samples; the distribution of C. *albicans* together with C. *glabrata* was identical to that in a study by Ng et al.[25] There was a spectrum change from *Candida albicans* to non-Albican *Candida* (NAC); this is compatible with a study by Ghazi et al.[26] C. dubliniensis represented 10% of isolates, exceeding C. *tropicalis*, C. *krusei*, and C. *parapsilosis*. There were differences in the species spectrum from other studies.[27,28] This is due to the rise in the epidemiology of C. *dubliniensis* during recent years.[29]

Data gathered and published by numerous sentinel and population-based surveillance projects have greatly improved our knowledge about the incidence of invasive fungal diseases and related resistance and susceptibility profiles.[30–32] In the current study, we suggested the in vitro susceptibility testing profiles of six antifungals for nine *Candida* species. We identified 42 (42%) isolates among *Candida* species with limited susceptibility to 5-FC, MIC > 0.5 µg/mL was used to designate the flucytosine non-wild type of *Candida albicans*, and other species in the study except C. krusei, an ECV of MIC > 32 µg/mL was used to characterise the flucytosine non-wild type of *Candida krusei*.[22] As we know, this is the first article explaining the patterns of susceptibility to 5-FC against *Candida* species in Iraq. This susceptibility pattern to 5-FC disagrees with the in vitro activity of the drug in other studies.[33,34] C. *albicans* remained the species with the highest fluconazole susceptibility rate at 92.5% (50/54) followed by C. *dubliniensis* at 90% (9/10), C. *tropicalis* at 50% (3/6), and C. *glabrata* at 100% (20/20). For C. *albicans*, 5-FC presented the most elevated geometric mean MIC value (3.97), then FLC (3.28), VRC (0.85), AMB (0.75), ITR (0.24), and CAS (0.17).

In our study, C. *tropicalis* together with C. *parapsilosis* isolates are resistant to FLC and ITR (100%), and both were susceptible to VRC. Among C. *tropicalis*, six isolates showed resistance to FLC and three to VRC, and all were susceptible to ITR. This in vitro activity of these two species is compatible with other studies in Asia. C. *tropicalis* and C. *parapsilosis* were reported as the Candida species with the maximum elevated MIC values in the region other than C. *albicans*.[35,36] We reported that C. *glabrata* intrinsically displayed a reduced susceptibility to FLC. All twenty sample cultures of the species demonstrated an elevation in MIC values (1–16 µg/mL) for fluconazole. The reduced susceptibility was also noticed in seven (35%) isolates for AMB. This result was compatible with a study in Kuwait.[37] Out of our 10 isolates of C. *dubliniensis*, two displayed reduced susceptibility to ITR and AMB and only 1 isolate for VRC, FLC, and 5-FC. Despite the reduced susceptibility, C. *dubliniensis* is still considered sensitive to these drugs and showed full susceptibility to CAS. This result was compatible with that of a study by Khan et al.[38] C. krusei also displayed full susceptibility to all six tested antifungals, except one sample, which presented decreased susceptibility to AMB. These results were compatible with the Khalifa et al. study in Japan, except for CAS, which showed the opposite of our findings.[39]

The non-susceptible rate to the six antifungal agents was 5-FC (42%), FLC (21% were I) (9% were R), AMB (11%), ITR (8%), VRC (6%), and CAS (4% were I) (1% were R). We noticed that the reduced susceptibility rate was increased for 5-FC, FLC, ITR, AMB, and VRC. The reason relied on the massive consumption of corticosteroids and strong antibiotics with a broad spectrum after the pandemic of COVID-19, a respiratory disease that appeared in 2019 for the first time, especially in our isolates, which were mostly respiratory (76%).[40,41]

Conclusion

In conclusion, we have documented the *Candida* isolates antifungal susceptibility profile to six antifungal agents, with analysis based on the updated CLSI breakpoints. There has been an elevated resistance to antifungal drugs except for

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caspofungin. C. *albicans* showed an increased 5-FC non-WT phenotype (72%), with elevated MIC values, while C. *glabrata* showed a high percentage of non-WT (7%) against AMB. Also, C. *tropicalis* together with C. *parapsilosis* are less susceptible to azoles. Therefore, checking for antifungal susceptibility is suggested before treating candidiasis, especially for C. *albicans*, C. *tropicalis*, C. *glabrata*, and C. *parapsilosis*.

Further studies and follow-ups are important in Iraq to track the incidence trends, emergence of the species, and antifungal medication profiles required to inform the therapeutic process of candidiasis.

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