Egypt. J. Microbiol. **Vol. 58,** pp. 11- 23 (2023)

EgyptianJournalofMicrobiology
http://ejm.journals.ekb.eg/

Analysis of Species Distribution and Antifungal Susceptibilities among Locally Prevailed Clinical Isolates of *Candida*

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GLOBALLY, *Candida* species are the most prevalent cause of invasive mycotic infections, which are associated with high mortality rates and exhibit remarkable geographic and epidemiological diversity. A better understanding of *Candida*-associated infections' epidemiology and species incidence is needed. This study analyzed *Candida* species distribution and antifungal susceptibilities in 200 locally collected clinical specimens. Isolates recovered were identified using the standard conventional methods and MALDI-TOF-MS; susceptibility testing was run for Fluconazole, Itraconazole, Voriconazole, Posaconazole, Caspofungin, Amphotericin-B, and Nystatin, according to the CLSI M44-A2 guideline. *C. albicans* accounted for 92(48.9%) among 188 *Candida* isolates obtained. In contrast, species distribution among the non-albicans *Candida* isolates (no.= 96) was as follows: *C. parapsilosis* (no. =35), *C. krusei* (no. =21), *C. tropicalis* (no. =20), and *C. glabrata* (no. =20). In collected clinical specimens, *Candida* species were significantly more common in urine (64.4%), sputum (18.1%), and blood (10.1%). *C. albicans* was the predominant species in all types of clinical samples except in blood, where *C. parapsilosis* dominates. The isolated *Candida* species showed variable resistance rates to the seven tested antifungals. Fluconazole resistance was the most common (56/188 isolates); 44% of NAC isolates were FLC-resistant compared with 15.2% for isolated *C. albicans*. Disturbingly, cross-resistance to FLC and VRC was recorded in all VOR-resistant isolates of *C. krusei, C. parapsilosis,* and *C. glabrata*; moreover, eleven out of twelve VOR-resistant *C. albicans* isolates exhibited FLC-VRC cross-resistance.

Keywords: Antifungal resistance, *Candida* sp., Cross-resistance, Distribution, Egypt.

Introduction

Increased resistance rates point to the need for more attention regarding antifungal resistance growingly developed for many infections caused by *Candida* species, the most common cause of invasive mycotic infections. Resistance to antifungal agents is a clinical challenge and, when it exists, a primary factor for treatment failure in patients with invasive candidiasis. Invasive *Candida* infection is an increasing global threat with elevated mortality rates. It may affect all age groups, particularly cancer patients, immunosuppressed, critically ill patients in ICU, and people with diabetes mellitus or with uncontrolled high blood sugar that requires invasive mechanical ventilation (Bitar et al., 2014; Lortholary et al., 2014; McCarty & Pappas, 2016; Epelbaum & Chasan, 2017; Alves et al., 2018; Ding et al., 2018; Boonsilp et al., 2021). Invasive candidiasis affects more than 250,000 persons worldwide each year. Estimated death rates from this infection may be as high as 50% (Kullberg & Arendrup, 2015; Cornely et al., 2020). Such mortality rates are further increased in the context of host immunosuppression and infection with antifungal-resistant *Candida* isolates (Pappas et al., 2009, 2016). *Candida albicans* was the frontrunner in such infections, with little emphasis on non-albicans *Candida* species. However, a gradual shift to non-albicans *Candida* species (NACS), especially *C. parapsilosis, C. glabrata,*

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Received: 11/06/2023; Accepted: 20/08/2023
DOI: 10.21608/EJM.2023.216815.1230

C. auris, and *C. tropicalis*, occurred (Satoh et al., 2009; Arendrup, 2010; Friedman & Schwartz, 2019).

Triazoles, Echinocandins, and Polyene are the most frequently used antifungals for treating different *Candida* infections. For most *Candida* infections, Azoles (especially fluconazole) and Echinocandins are usually used as first-line therapy (Pfaller et al., 2010; Pathadka et al., 2022). All can be used with varying efficiency depending on the infection type and site and the infecting isolate's susceptibility. Antifungal susceptibility pattern fluctuates across different *Candida* species. The inception and spread of isolates' resistance to commonly used drugs leading to treatment failures are being increasingly reported. The latter may be attributed to the increase in infections caused by NAC species, often intrinsically less susceptible or resistant to antifungal agents, particularly fluconazole and echinocandins, along with the problem of acquired resistance, which is strongly motivated by the overuse of antifungals for curative and prophylactic purposes (Ksiezopolska & Gabaldón, 2018; Mesini et al., 2020).

Most global surveillance programs imply substantial differences in the distribution and antifungal susceptibility of many *Candida* species over time and geographic area (Pfaller et al., 2004, 2007, 2010; Castanheira et al., 2020). Although a better understanding of epidemiological information regarding species distribution and drug resistance of invasive *Candida* infection in the healthcare sector is intrinsic in establishing treatment protocols, directing the choice of the right antifungal therapy, besides evaluating the potential impact of novel antifungal agents (Pfaller & Diekema, 2007; Warnock, 2007). The epidemiology of fungal diseases, including *Candida* infections, is inadequately understood in Egypt, where most of these diseases are either under-reported or not reported at all. Furthermore, like many developing countries, healthcare settings ignore investigations that assess the prevalence and etiology of fungal infections, especially among high-risk patients.

The present study was designed to identify and figure out the species distribution of locally prevailed *Candida* isolates; and to determine antifungal susceptibility patterns of *Candida* species to seven antifungal agents, viz., Fluconazole, Itraconazole, Voriconazole, Posaconazole, Caspofungin, Amphotericin-B, and Nystatin. Moreover, cross-resistance profiles among the studied *Candida* isolates were determined.

Methods

Sample collection

Twohundred suspected *Candida* cultures were supplied by the Clinical Microbiology Laboratory of a Governmental Hospital in Cairo, Egypt, between January 2017 and June 2018. These cultures were isolated from 200 different clinical samples received in the laboratory from various hospital departments to confirm *Candida* infection. Cultures were then transferred to the Mycology research laboratory, Department of Microbiology, Faculty of Science, Ain-Shams University, to identify isolates. A part of each culture was subjected to direct microscopy and Gram stain for investigation of yeast cells and pseudohyphae. Suspected *Candida* colonies were purified by sub-culturing onto Sabauraud›s dextrose agar (SDA; Difco, Sparks, MD, USA) supplemented with chloramphenicol for 24 - 48h at 37°C. Isolates were stored at - 20°C in 20% glycerol for future antifungal susceptibility tests and identification (Bergman, 2001).

Candida species identification

Isolates were phenotypically identified to the species level using standard conventional methods. Chromogenic medium (CHROMagar™ *Candida*, Paris, France) was used according to the manufacturer's instructions. All isolates were examined by the germ tube assay via inoculating colonies in human serum (0.5mL) for three h of incubation at 37°C. Then, the Dalmau plate technique was performed according to McGinnis (1980). Identification was confirmed using Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF, Bruker-Daltonics, Bremen, Germany).

Antifungal susceptibility testing

The antifungal susceptibility pattern of the obtained isolates of *Candida* sp. was examined using the disk diffusion susceptibility test as described by Berkow et al. (2020) according to the guideline of the Clinical and Laboratory Standards Institute (CLSI, M44-A2, 2009). Commercially available antifungals: Fluconazole (FLU; 25mcg/Disk), Voriconazole (VOR; 1mcg/Disk), Nystatin (NY; 100mcg/Disk), Posaconazole (POS; 5mcg/Disk), Itraconazole (ITR; 10mcg/

Disk), Caspofungin (CAS; 5mcg/Disk) were purchased from Bioanalyse ® limited, Ankara/ Turkey. Whereas Amphotericin B (AMB; 20mcg/ Disk) was obtained from Liofilchem ® s.r.l, Italy. According to manufacturers› instructions, the discs were stored in the refrigerator (2 – 8°C). Before use, discs were placed at room temperature for one h to reduce condensation resulting from the reaction with warm air. To standardize the discs› potency, they were tested against four reference strains, including *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, and *C. tropicalis* ATCC750. Colonies were suspended in 5mL of sterile 0.85% saline before adjusting turbidity to a yield of 1×105 - 1×106 CFU/mL (0.5 McFarland standard). A sterile cotton swab was dipped into the suspension before inoculation onto surfaces of Muller-Hinton agar (MHA; Difco, Sparks, MD, USA) plates. All plates were dried for 3-5 min at room temperature, under aseptic conditions. Antifungal discs were placed on the inoculated plates; following diffusion, plates were incubated at 37°C. Inhibition zones were recorded after 24h. and 48h. Results were interpreted as follows: resistant (R) or sensitive (S) and susceptible dose-dependent (SDD) according to the Zone diameter interpretive standards for Antifungal Disk Diffusion Susceptibility Testing of *Candida* sp., depending on the manufacturers' instructions.

Statistical analysis

IBM SPSS statistics (V. 26.0, IBM Corp., USA, 2019) was used for data analysis. Pearson's chi-square test was performed to analyze differences in distribution and frequencies among variables. Calculated Relative Risk Assessments (Relative Risk Ratio or RRR) were determined as absolute figures and as a standard error of estimate (95 %). The probability of error (P-value) at 0.05 was considered statistically significant., while 0.01 and 0.001 are highly significant.

Results

The distribution of *Candida* species concerning source and type of clinical samples and Pre-Disposing Risk Factors are presented in Fig. 1 and Table 1. A total of 188 isolates of *Candida* species were isolated from 200 different clinical specimens. Isolates recovered were: 121 (64.4%) from urine, 34(18.1%) from sputum, 19 (10.1%) from blood, 9 (4.8%) from swabs from contaminated wounds, 3 (1.6%) from vaginal swabs, and 2 (1.1%) from swabs from chest wounds.

Of the 188 positive samples, 176 accounting for more than 90%, were obtained from patients with comorbidities and/or risk factors for developing *Candida* infection. Diabetes mellitus and malignancy were the most common comorbidities, reported in 93 (49.5%) and 27 (14.4%) patients, respectively. Noteworthy, 34 (18.1%) patients were on previous antibiotic medication. The chi-squared test revealed a non-statistically significant difference in the incidence of *Candida* species analyzed according to pre-disposing risk factors (P= 0.683).

The isolation rate of non-albicans *Candida* species was slightly higher (51.1%) compared to *C. albicans* (48.9%). Among NAC species, *C. parapsilosis* was the most dominant species (18.6%), followed by *C. krusei* (11.2%), *C. tropicalis* (10.6%), and *C. glabrata* (10.6%). *C. albicans* dominates in all types of studied clinical specimens except in blood (26.3%), where *C. parapsilosis* was predominant (47.4%). For urine samples, 68 out of 122 (56.2%) were NAC species, while 53 (43.3%) were *C. albicans*. The most prevailing among NAC isolated from urine was *C. parapsilosis* (20122/), whereas the least was *C. krusei* (12122/), with isolation frequencies of 16.4% and 9.8%, respectively. In analyzed sputum samples, the most frequent species was *C. albicans* (67.6%), while the least was *C. tropicalis* (only 2.9%). Of note, *C. albicans* was the only species isolated from vaginal and chest wound swabs (Table 1). Statistically significant differences were detected in the distribution of *Candida* species according to the specimen type (P = 0.008).

In vitro susceptibility pattern for the 188 collected *Candida* isolates during the study period assessed towards seven antifungals is illustrated in Table 2 and Fig. 2. Concerning azole antifungals, posaconazole showed good activity and was mostly effective against most tested *Candida* species. Susceptibility percentages to posaconazole were: 100%, 96.7%, 90.5%, 85.0, and 75.0% recorded for *C. parapsilosis, C. albicans, C. krusei, C. tropicalis,* and *C. glabrata*, respectively. Of concern was the reduced effect of fluconazole (56 fluconazole-resistant/188 isolates).

TABLE 1. Influence of pre-disposing risk factors on *Candida* species distribution

Comorbidities /risk factors	No. of isolated *Candida* species						Total No. (%)
	C. albicans	Non-albicans *Candida*	*C. glabrata*	*C. krusei*	*C. parapsilosis*	*C. tropicalis*	
Diabetes mellitus	41	52	12	9	16	15	**93(49.5%)**
Prior antibiotic administration (AB)	18	16	1	5	10	0	**34(18.1%)**
Cancer	12	15	3	4	5	3	**27(14.4%)**
Diabetes +AB	1	2	1	1	0	0	**3(1.6%)**
Cancer +AB	3	2	0	0	2	0	**5(2.7%)**
Diabetes + cancer	2	0	0	0	0	0	**2(1.0%)**
Diabetes+ cancer +AB	0	1	0	0	0	1	**1(0.5%)**
Diabetes + renal failure	1	0	0	0	0	0	**1(0.5%)**
Renal failure	0	1	0	0	0	1	**1(0.5%)**
Cancer +renal failure	1	0	0	0	0	0	**1(0.5%)**
Urinary bladder abnormalities	4	0	0	0	0	0	**4(2.1%)**
Failure in respiration	0	1	0	0	1	0	**1(0.5%)**
Open heart	2	0	0	0	0	0	**2(1.0%)**
Surgery+ cancer	1	0	0	0	0	0	**1(0.5%)**
Non (Normal)	6	6	3	2	1	0	**12(6.4%)**
Total No. (%)	**92(48.9%)**	**96 (51.1%)**	**20(10.6%)**	**21(11.2%)**	**35(18.6%)**	**20(10.6%)**	**188(100%)**

Chi-square test revealed that: there was a non-statistically significant difference in the incidence of *Candida* spp. analyzed according to pre-disposing risk factors (P = 0.683).

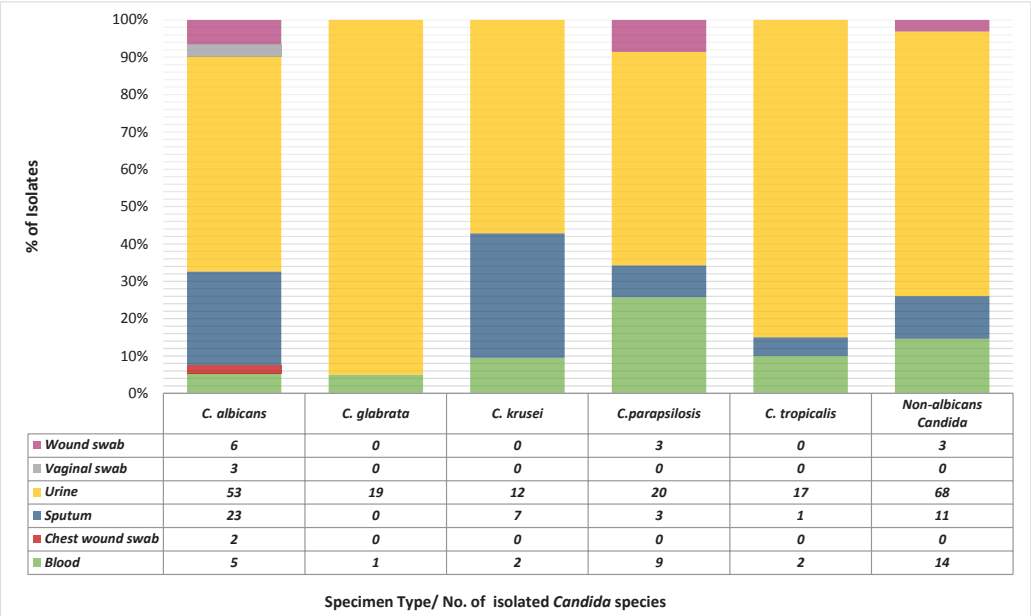
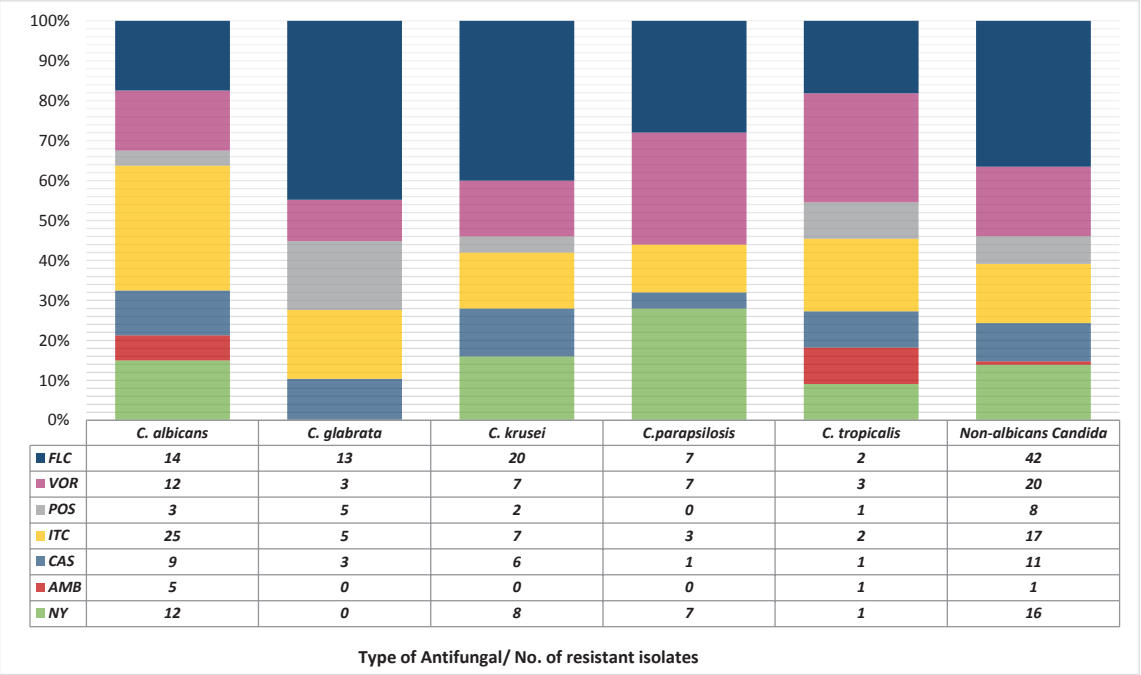


Fig. 1. Distribution of *Candida* species among various clinical specimen, (total No. = 188 isolates)

TABLE 2. ***In vitro*** **susceptibility profile of isolated** ***Candida*** **species to seven antifungal agents**

Antifungal	*Candida* species (No. of isolates)	No. of isolates (% of the species)				OR (95% CI) (Minimum-Maximum)	P-value
		S	SDD	Total susceptibility	R		
FLC	***C. albicans (92)***	73(79.3%)	5(5.4%)	**78(84.8%)**	**14(15.2%)**	0.23 (0.11- 0.46)	< 0.001
	C. glabrata (20)	2(10%)	5(25%)	**7(35%)**	**13(65%)**	5.39 (2.02- 14.41)	
	C. krusei (21)	1(4.8%)	0 (0.0%)	**1(4.8%)**	**20(95.2%)**	72.77 (9.44- 5.60)	
	C. parapsilosis (35)	25(71.4%)	3(8.6%)	**28(80%)**	**7(20%)**	0.53 (0.21- 1.29)	
	C. tropicalis	16(80%)	2(10%)	**18(90%)**	**2(10%)**	0.23 (0.05- 1.04)	
VRC	***C. albicans (92)***	75(81.5%)	5(5.4%)	**80(87%)**	**12(13%)**	0.57 (0.26- 1.24)	0.424
	C. glabrata (20)	14(70%)	3(15%)	**17(85%)**	**3(15%)**	0.84 (0.23- 3.07)	
	C. krusei (21)	13(61.9%)	1(4.8%)	**14(66.6%)**	**7(33.3%)**	2.84 (1.04- 7.73)	
	C. parapsilosis (35)	26(74.3%)	2(5.7%)	**28(80%)**	**7(20%)**	1.28 (0.50- 3.25)	
	C. tropicalis (20)	16(80%)	1(5%)	**3(15%)**	**3(15%)**	0.84 (0.23- 3.07)	
POS	***C. albicans***	88(95.7%)	1(1.1%)	**89(96.7%)**	**3(3.3%)**	0.37 (0.09- 1.44)	< 0.001
	C. glabrata (20)	12(60%)	3(15%)	**15(75.0%)**	**5(25%)**	9.00 (2.15- 33.00)	
	C. krusei (21)	17(81%)	2(9.5%)	**19(90.5%)**	**2(9.5%)**	1.84 (0.37- 9.19)	
	C. parapsilosis (35)	34(97.1%)	1(2.9%)	**35(100%)**	**0 (0.0%)**	-	
	C. tropicalis (20)	16(80%)	3(15%)	**19(85.0%)**	**1(5.0%)**	0.83 (0.10- 6.85)	
ITC	***C. albicans (92)***	62(67.4%)	5(5.4%)	**67(72.8%)**	**25(27.2%)**	0.25 (0.13- 0.47)	< 0.001
	***C. glabrata* (20)**	12(60%)	3(15%)	**15(75.0%)**	**5(25%)**	9.00 (2.15- 33.00)	
	C. krusei (21)	13(61.9%)	1(4.8%)	**14(66.6%)**	**7(33.3%)**	2.84 (1.04- 7.73)	
	C. parapsilosis (35)	16(80%)	1(5%)	**17(85%)**	**3(15%)**	0.84 (0.23- 3.07)	
	C. tropicalis (20)	16(80%)	2(10%)	**18(90%)**	**2(10%)**	0.23 (0.05- 1.04)	
CAS	***C. albicans (92)***	83(90.2%)	0 (0.0%)	**83(90.2%)**	**9(9.8%)**	0.83 (0.32-2.10)	< 0.001
	***C. glabrata* (20)**	17(85%)	0 (0.0%)	**17(85%)**	**3(15%)**	1.67 (0.44-6.30)	
	C. krusei (21)	15(71.4%)	0 (0.0%)	**15(71.4%)**	**6(28.6%)**	4.34 (1.45-12.96)	
	C. parapsilosis (35)	34(97.1%)	0 (0.0%)	**34(97.1%)**	**1(2.9%)**	0.31 (0.04-2.45)	
	C. tropicalis (20)	19(95%)	0 (0.0%)	**19(95%)**	**1(5%)**	0.41 (0.05-3.23)	
AMB	***C. albicans***	45(48.9%)	42(45.7%)	**87(94.6)**	**5(5.4%)**	0.65 (0.36- 1.16)	< 0.001
	C. glabrata (20)	4(20%)	16(80%)	**18(100%)**	**0(0%)**	-	
	C. krusei (21)	13(61.9%)	8(38.1%)	**21(100%)**	**0(0%)**	-	
	C. parapsilosis (35)	22(62.9%)	13(37.1%)	**35(100%)**	**0(0%)**	-	
	C. tropicalis (20)	18(90%)	1(5%)	**19(95.0%)**	**1(5%)**	9.00 (2.02- 40.00)	
NY	***C. albicans (92)***	35(38%)	45(48.9%)	**80(87.0%)**	**12(13%)**	0.53 (0.24- 1.16)	0.014
	C. glabrata (20)	2(10%)	18(90%)	**20(100%)**	**0 (0.0%)**	-	
	C. krusei (21)	0 (0.0%)	13(61.9%)	**13(61.9%)**	**8(38.1%)**	2.08 (0.80- 5.41)	
	C. parapsilosis (35)	5(14.3%)	23(65.7%)	**28(80.0%)**	**7(20%)**	0.73 (0.29- 1.80)	
	C. tropicalis (20)	3(15%)	16(80%)	**19(95.0%)**	**1(5%)**	0.14 (0.01- 1.10)	

Susceptibility categories were determined using CLSI M44-A2, FLC, Fluconazole; VRC, Voriconazole; ITC, Itraconazole; POS, Posaconazole; CAS, Caspofungin; NY,-Nystatin; AMB, Amphotericin B; S, susceptible; SDD: susceptible dose-dependent; R, resistant.; OR: odds ratio; CI: confidence interval; *P*-value <0.05 is considered significance, P-value <0.001 is highly significant.



	C. albicans	C. glabrata	C. krusei	C.parapsilosis	C. tropicalis	Non-albicans Candida
FLC	14	13	20	7	2	42
VOR	12	3	7	7	3	20
POS	3	5	2	0	1	8
ITC	25	5	7	3	2	17
CAS	9	3	6	1	1	11
AMB	5	0	0	0	1	1
NY	12	0	8	7	1	16

Fig.2. Rate of resistance of the isolated *Candida* species to seven tested antifungals [FLC, Fluconazole; VRC, Voriconazole; ITC, Itraconazole; POS, Posaconazole; CAS, Caspofungin; AMB, Amphotericin; NY, Nystatin]

For *C. albicans*, the highest resistance was observed to itraconazole (27.2%), fluconazole (15.2%), and voriconazole (13.0%). Non-albicans *Candida* (NAC) isolates were generally more resistant to fluconazole and voriconazole, with recorded values of 43.8% and 20.8%. Among the studied *NAC* species, *C. krusei* scored the highest resistance rate to fluconazole at 95.2%, and *C. tropicalis* scored the lowest by 10% to the same azole. Interestingly, as shown in Table 3, most of the isolates that showed resistance to voriconazole were cross-resistant to fluconazole (29/32 isolates). All voriconazole-resistant isolates of *C. krusei* (7 isolates), *C. parapsilosis* (7 isolates) and *C. glabrata* (3 isolates), were cross-resistant to fluconazole; moreover, cross-resistance to voriconazole and fluconazole was observed in 11 out of 12 voriconazole-resistant *C. albicans* isolates. Little cross-resistance was detected among the other tested triazoles.

Echinocandin antifungal «Caspofungin» revealed good activity against isolates of *C. parapsilosis* with 97.1% susceptibility, followed by *C. tropicalis* (95%). In contrast, lower susceptibilities were recorded for *C. krusei* (71.4%), *C. glabrata* (85%), and *C. albicans* (90.2%). Regarding the polyene antifungals, Amphotericin B exhibited a very good activity pattern against all *Candida* species with 100% susceptibility for isolates of *C. glabrata, C. krusei,* and *C. parapsilosis,* and 95% for *C. albicans* and *C. tropicalis.* All *C. glabrata* isolates were highly susceptible to the topical polyene antifungal «Nystatin». In contrast, lower susceptibility was recorded for *C. tropicalis, C. albicans, C. parapsilosis,* and *C. krusei,* which reached 95%, 87%, 80%, and 62%, respectively (Table 2).

Discussion

Recently, there has been significant geographic and population variation in the predominance of pathogenic *Candida* sp. (Taei et al., 2019; Xiao et al., 2020; Boonsilp et al., 2021). *C. albicans* is still a dominant agent of invasive candidiasis, but species distribution has gradually changed to non-albicans *Candida* species (NACS) (Arendrup, 2010; Friedman & Schwartz, 2019). Given this ever-evolving epidemiological landscape of candidiasis worldwide and the emergence of NAC often associated with treatment failure, continuous monitoring is essential, specifically in under-investigated geographic regions like Egypt. Hence, the present work was planned to inspect the species distribution and explore the prevalence of causative *Candida* species and their antifungal susceptibility profiles.

TABLE 3. Cross-resistance to Fluconazole with Voriconazole and other azole drugs

Candida species (No. of isolates)	No. of isolates (% of the species)				
	Resistance to FLC	**Resistance to VRC**	**Cross-resistance to FLC, VRC**	**Cross-resistance to FLC, VRC, POS**	**Cross-resistance to FLC, VRC, POS, ITC**
C. albicans (92)	14(15.2%)	12(13%)	11(11.9%)	2(2.2%)	2(2.2%)
C. glabrata (20)	13(65%)	3(15%)	3(15%)	2(10%)	2(10%)
C. krusei (21)	20(95.2%)	7(33.3%)	7(33.3%)	1(4.8%)	0 (0.0%)
C. parapsilosis (35)	7(20%)	7(20%)	7(20%)	0 (0.0%)	0 (0.0%)
C. tropicalis (20)	2(10%)	3(15%)	1(5%)	0 (0.0%)	0 (0.0%)
Total (188)	**56 (29.8%)**	**32 (17.1%)**	**29 (15.4%)**	**5(2.7%)**	**4(2.1%)**

FLC, fluconazole; VRC, voriconazole; ITC, itraconazole; POS, posaconazole;

Five different *Candida* species were identified in this investigation, and in line with the global trends of increasing rates of NACs, the species distribution among candidiasis patients showed a higher frequency of NAC species (51.1%) than *C. albicans* (48.9%). This seems consistent with several previous investigations in the Middle East and North African countries, where the recorded incidence of NAC species was 55%, 56.2%, 65.9%, 66.2%, 68.4 %, and 69% in the United Arab Emirates, Turkey, Saudi Arabia, Qatar, Algeria, and Kuwait, respectively (Ellis et al., 2003; Kazak et al., 2014; Al Thaqafi et al., 2014; Taj-Aldeen et al., 2014; Arrache et al., 2015; Yeşilkaya et al., 2017; Alobaid et al., 2021). The present results comply with recently published reports in Egypt, first by El-Mashad et al. (2019) and then by Reda et al. (2023), in which the majority of *Candida* infections were caused by NAC species that accounted for 59.8% and 51.6%, respectively. Conversely, an Egyptian study reported that *C. albicans* was the most commonly isolated species, with an isolation rate of 57.4%, compared to 42.6% for all NACs (El-Ganiny et al., 2021).

Of all *Candida* species isolated in this study, *C. albicans* remained the most frequent (48.9%). This is consistent with previous records for *C. albicans* as a dominant cause of *Candida* infections in different geographic regions, including Italy (61.2%), Saudi Arabia (38.3%), China (36.1%), Kuwait (31%), and Egypt (27.8% in adults, and 48.3% in pediatrics) (Lin et al., 2018; Mencarini, 2018; Al-Dorzi et al., 2020; Alobaid et al., 2021; Reda et al., 2023). Results of our study highlighted that the most commonly isolated NACs were *C. parapsilosis* (18.6%), followed by *C. krusei* (11.2%), *C. tropicalis* (10.6%), and *C. glabrata* (10.6%). In line with our findings, some studies conducted in the Middle East detected the domination of *C. parapsilosis* among NAC species, including Iran (17.5%) and Kuwait (22.6%), as well as in Egypt (25%) (Hegazi et al., 2014; Sadrossadati et al., 2018; Alobaid et al., 2021).

In contrast to the present results, *C. tropicalis* was the predominant cause of NAC candidiasis, followed by *C. parapsilosis, C. tropicalis, C. glabrata,* and *C. krusei*, as recorded in Egypt by Reda et al. (2023). While in another Egyptian report conducted by Abass et al. (2019), *C. krusei* was the most prevalent NAC species among ICU patients, followed by *C. parapsilosis, C. tropicalis,* and *C. glabrata*. Such differences could be due to several aspects, including infection type, sample size, patient population, existence of risk factors, and study duration. The high incidence of *C. krusei* (11.2%) and *C. glabrata* (10.6%) observed in this study could be due to the excessive use of the antifungal fluconazole; this may be supported by Antinori et al. (2016), who reported that treatment of mycotic infections with azoles, including fluconazole, can promote the election of resistant species of *Candida* by shifting infection toward intrinsically more resistant species of *C. krusei* and *C. glabrata.*

In vitro susceptibility tests for *Candida* isolates towards tested antifungals revealed variable resistance patterns. For Azole antifungals, resistance to fluconazole was the most common (56 /188 isolates; 29.8%), fluconazole remains the most widely used antifungal agent, particularly in countries of low and middle-income,

including Egypt, and this may explain elevation in fluconazole resistance reported in this study. It was previously reported that extensive use of fluconazole leads to the emergence of resistance in all *Candida* species (Pfaller et al., 2010; Whaley et al., 2017; Pathadka et al., 2022).

Around half of NACs isolates were significantly resistant to fluconazole, whereas 15.2 % of *C. albicans* isolates were resistant to this azole. Variations in azole-resistance have been recorded in several recent studies among *C. albicans*. Three research articles by Elnagar et al. (2023), Zakhem et al. (2021), and File et al. (2023) reported that 25.3%, 12.5%, and 7.3%, respectively, of *C. albicans* isolates were fluconazole-resistant. *Among* the NACs isolated here, *C. krusei* showed the highest resistance to fluconazole (95.2%), followed by *C. glabrata* (65%). This is in parallel to many reports published by Abbas et al. (2019), Pfaller et al. (2010), and Zakhem et al. (2021). *C. krusei* is known to be intrinsically resistant to fluconazole; the inherent low sensitivity recorded in *C. glabrata* to fluconazole and other azoles has also been reported. It was found that *C. glabrata* has the potential to acquire a high resistance after exposure to azole treatment (Pfaller et al., 2008; Lamping et al., 2009; Pfaller et al., 2014; Whaley et al., 2017).

About 20% of *C. parapsilosis* showed resistance to fluconazole; this finding was similar to those described in many previous reports (Castanheira et al., 2020; Mesini et al., 2020; Demirci-Duarte et al., 2021; Mamali et al., 2022). Of note, fluconazole resistance was once uncommon among isolates of *C. parapsilosis*, but recently, an ever-increasing number of fluconazole-resistant clinical isolates of *C. parapsilosis* has been recorded worldwide (Mesini et al., 2020). Cross-resistance to voriconazole and fluconazole occurred in a significant number of studied isolates, where all voriconazole-resistant isolates of *C. krusei* (7 isolates), *C. parapsilosis* (7 isolates) and *C. glabrata* (3 isolates), were proved to have Cross-Resistance to fluconazole. Furthermore, FLC-VRC Cross-Resistance was also detected in eleven out of twelve voriconazole-resistant *C. albicans* isolates. This finding is in line with some previous reports by Astvad et al. (2018), Mete et al. (2021), and Mamali et al. (2022).

Echinocandins are a class of antifungal agents that have been available for more than two decades and are now recommended as a first-line treatment for invasive candidiasis, especially against azole-resistant *Candida* isolates (Cornely et al., 2012; Pappas et al., 2016). The expanded use of echinocandins, including caspofungin, has increased the number of breakthrough infections attributed to multidrug-resistant *Candida* species, which are mainly resistant to azoles (Yang et al., 2017; Mroczyńska & Brillowska-Dąbrowska, 2020; Perrine-Walker, 2022). In the present study, caspofungin showed good activity when tested for isolates of *C. parapsilosis* (34 isolates out of 35 were susceptible) and *C. tropicalis* (19 isolates out of 20 were susceptible), where only a single isolate of both species where resistant to this echinocandin antifungal with resistance rates comprising 2.9% and 5%, respectively. However, higher rates of resistance were recorded for others, including *C. krusei* (6 resistant isolates out of 21*;* 28.6%), *C. glabrata* (3 /20 isolates; 15%), and *C. albicans* (9 /92 isolates; 9.8%). In general, documented records of resistance to echinocandin remain relatively low, at <3% in most *Candida* species, except for *C. glabrata* (Perlin, 2015). While in agreement with the present results, a higher level of resistance among *C. krusei* isolates has been recorded by few reports. (Pfaller et al., 2008; Tavernier et al., 2015). Mutations in ERG11 and FKS 1 gene are the most common mechanisms of resistance to both azole and echinocandin antifungals in *Candida* species, including *C. krusei* (Forastiero et al., 2015; Feng et al., 2016; Perlin et al., 2017). Moreover, the resistance of *C. krusei* can also be explained by the over-expression of the efflux pump mechanism (Lamping et al., 2009; Gong et al., 2018).

Elevated echinocandin resistance was frequently reported among clinical isolates of *C. glabrata* (Pfaller et al., 2012; Alexander et al., 2013; Pham et al., 2014). In consistence with our findings (15% of *C. glabrata* isolates were resistant to caspofungin), a 10-year study in England recorded that echinocandin resistance of *C. glabrata* isolates was increased from about 4% to >13% in the period of experiment between 2001 and 2010 (Alexander et al., 2013). Regarding *C. albicans*, although it is uncommon, several reports documented resistance to caspofungin in *C. albicans*, in line with our findings (Yang et al., 2017; De-Cesare et al., 2022; PerrineWalker, 2022).

Although *Candida* species rapidly develop

resistance to echinocandin and azole antifungals, resistance to amphotericin B is still sporadic despite decades of use for this old antifungal (Vincent et al., 2013). All species reported here were generally sensitive to amphotericin B. It should be noted that while this drug is not a first-line therapeutic agent of invasive candidiasis in most clinical settings, amphotericin B may have a vital role when the azoles and/or echinocandins can't be used, particularly as the number of isolates resistant to these antifungals increases, or in rescue therapy (Pappas et al., 2009, 2016).

Conclusion

These findings help us understand at least part of the reason for differences in the epidemiology associated with establishing some *Candida* species in Egypt. For example, it was less surprising that non-albicans *Candida* were the dominant species. Also, a slight change in epidemiology was linked to the high and increased proportion of fluconazole-resistant isolates. Moreover, cross-resistance to voriconazole and fluconazole was significantly verified in a number of studied isolates, and this appears mostly to be related to the increased use of these antifungal drugs; this highlights the need for urgent monitoring of the local burden of antifungal resistance, which can prevent undermining of the clinical efficiency of commonly used antifungal agents and their negative consequences that may lead to treatment failure, more extended hospitalization, and increased costs. Finally, the above-mentioned local epidemiological data may be an essential step toward developing optimal treatment strategies and avoiding inappropriate treatment.

Ethics statement: The study was approved by the Research Ethics Committee of Faculty of Science, Ain-Shams University (ASU-SCI/MICR/2023/3/1).

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تحليل لتوزيع الأنواع وأنماط الحساسية لمضادات الفطريات بين عزلات الكانديدا السريرية السائدة محليًا

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على الصعيد العالمي، تعد أنواع الكانديدا هي السبب الأكثر شيوعاً لإنتشار العدوى الفطرية الغازية، والتي تصاحبها معدلات وفيات عالية وتظهر تنوعًا جغرافيًا ووبائيًا ملحوظ. وهناك حاجة ماسة إلى فهم أفضل لوبائيات العدوى المرتبطة بالكانديدا ومعدل حدوث الإصابة بأنواعها المختلفة. لذا فقد قامت هذه الدراسة بتحليل كلاً من توزيع الأنواع والحساسية لمضادات الفطريات بين العزلات السريرية من الكانديدا التي تم جمعها محليًا أثناء فترة الدراسة. حيث تم عزل أنواع الكانديدا من 200 عينة سريرية مختلفة من إحدى المستشفيات العامة. وقد تم التعرف على الأنواع باستخدام طرق التعريف التقليدية كما تم تأكيد التعريف باستخدام تقنية MALDI-TOF-MS؛ وتم إجراء اختبار الحساسية للفلوكونازول، والإيتراكونازول، والفوريكونازول، والبوزاكونازول، والكاسبوفونجين، والأمفوتريسين-بي، والنيستاتين، وفقًا لإرشادات CLSI M44-A2. هذا وقد تبين من النتائج عزل إجمالي 188 عزلة من الكانديدا من العينات السريرية التي تم الحصول عليها خلال الدراسة، حيث شكلت عزلات كانديدا البيكانس (92 عزلة) نسبة 48.9٪ منها. في المقابل، كان توزيع الأنواع بين عزلات الكانديدا غير البيضاء (non-albicans *Candida*) الست وتسعون على النحو التالي: كانديدا بارسيلويسز (35) ، كانديدا كروزاى (21) ، كانديدا تروبيكالس (20) ، كانديدا جلابيراتا (20). فيما يتعلق بنوع العينات السريرية ، كانت أنواع الكانديدا أكثر شيوعًا في عينات البول (64.4٪) تليها عينات البلغم (18.1٪) وعينات الدم (10.1٪). ومن بين جميع أنواع الكانديدا المعزولة، سادت الكانديدا البيكانس في جميع أنواع العينات السريرية باستثناء الدم حيث وجد أن كانديدا بارسيلويسز هى السائدة. وقد أظهرت أنواع الكانديدا المعزولة معدلات مقاومة متفاوتة لمضادات الفطريات السبعة التي تم اختبارها. بشكل عام كانت مقاومة الفلوكونازول هي الأكثر شيوعًا (56/188 عينة)، حيث أظهرت حوالي 44٪ من عزلات الكانديدا غير البيضاء (non-albicans *Candida*) مقاومة للفلوكونازول، بينما كانت 15.2٪ من عزلات الكانديدا البيكانس مقاومة للفلوكونازول. ومما يثير القلق أنه تم تسجيل مقاومة متصالبة (Cross-resistance) للفلوكونازول والفوريكونازول في جميع العينات المقاومة للفوريكونازول من عزلات كانديدا الكروزاى و كانديدا بارسيلوزيس وكانديدا جلابيراتا. كما أظهرت 11 عزلة من أصل 12 عزلة من الكانديدا البيكانس المقاومة للفوريكونازول مقاومة متصالبة للفلوكونازول والفوريكونازول.