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Original article

Phenotypic identification and antifungal susceptibility patterns of *Candida* species isolated from various clinical specimens in Suez Canal University Hospitals

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A R T I C L E   I N F O

A B S T R A C T

Background: *Candida* is the most common cause of fungal infections. *Candida* species are identified by different phenotypic methods. Accurate identification of *Candida* species enables appropriate selection of antifungal agents by clinicians. Azoles are the most frequently used antifungal drugs to treat *Candida* infections. However**,** resistance among previously susceptible *Candida* species has emerged which made antifungal susceptibility testing crucial. **Aim**: This study aimed to phenotypically identify the different *Candida* species isolated from various clinical specimens in Suez Canal University Hospitals (SCUHs), and to assess their antifungal susceptibility patterns. **Method:** One hundred and five clinical specimens were collected from different departments in SCUHs. Isolates were identified as *Candida* by colony morphology on Sabouraud dextrose agar and Gram staining. *Candida* species were phenotypically identified using germ tube test, hypertonic Sabouraud broth, corn meal agar, chromogenic *Candida* agar, KB006 Hi*Candida* Identification Kit and Vitek 2 YST-ID system. Antifungal susceptibility to fluconazole, voriconazole and amphotericin B was done by disk diffusion method. **Results:** Prevalence of *Candida* was 54.3%. *C. tropicalis* was the most common species followed by *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. parapsilosis* and lastly *C. kefyr*. Only one strain was resistant to amphotericin B**.** Eight strains were susceptible dose dependent, and 2 were resistant to fluconazole. No resistance was detected to voriconazole. **Conclusions:** The prevalence of candidiasis is remarkable. Non-*albicans Candida* species (NACs) cause most of these infections. Fluconazole and amphotericin B showed low resistance rates. No resistance to voriconazole was reported in this study. Therefore, voriconazole could be more effective as empirical therapy than fluconazole and amphotericin B. Accurate identification of *Candida* species is essential for therapeutic and prognostic impact, appropriate selection of antifungal agents by clinicians and controlling the increase of resistant *Candida* strains.

Introduction

Candida is the most common cause of fungal infections worldwide. It mostly lives as a commensal on skin and mucous membranes. However, in some cases it can cause opportunistic infections especially when the integrity of the skin

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and mucous membranes is interrupted. In addition to superinfection, which is due to inhibition of normal flora by broad spectrum antibiotics or other immunocompromising conditions [1].

Candidiasis is an infection caused by *Candida*, mostly limited to the skin, nails and mucous membranes. However, it can cause serious systemic infections.

The genus *Candida* includes numerous species involved in human infections such as *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. krusei, C. lusitaniae, C. kefyr, C. guilliermondii* and *C. dubliniensis* [2].

Candida species are identified by different phenotypic methods including examination of their morphological features, analysis of their ability of carbohydrates assimilation and/or fermentation, and their ability of assimilating different nitrogen compounds [3].

Numerous antifungal classes are used to treat *Candida* infections; polyenes, azoles, echinocandins and allylamines. These antifungals are used with varying efficacy depending on the type, site of infection and the susceptibility of different *Candida* species. Azoles are the most frequently used antifungal drugs to treat *Candida* infections as they achieve high success rates even over short durations of therapy. However**,** resistance among previously susceptible *Candida* species has emerged following the expanded use of antifungal agents [4].

Antifungal susceptibility testing methods including broth dilution, disk diffusion and E test are now available with species-specific breakpoints developed by The Clinical and Laboratory Standards Institute (CLSI) for multiple antifungal agents [5].

The current study aimed to phenotypically identify the different *Candida* species isolated from various clinical specimens in Suez Canal University Hospitals (SCUHs), and to assess their antifungal susceptibility patterns.

Methodology

Study population

This cross-sectional descriptive study was conducted in SCUHs in Ismailia, Egypt, during the period from August 2021 to March 2022. One hundred and five clinical specimens were collected by simple random sampling technique from patients of different age groups in different departments in

SCUHs who had clinical suspicion of candidiasis and had not received antifungal drug therapy within 2 weeks before specimen collection. Both males and females were represented from all age groups. The ethics committee of Faculty of Medicine, Suez Canal University had approved the study.

Sample size justification

Sample size was calculated according to the following formula [6]:

$$n = \left[\frac{Z_{\alpha/2}}{E}\right]^2 * P(1 - P)$$

Where n= sample size. Z α/2 = 1.96 (The critical value that divides the central 95% of the Z distribution from the 5% in the tail). p = the prevalence of *Candida albicans* in clinical specimens =47.2 % [7]. E = the margin of error (width of confidence interval)

So, sample size equals 95 patients with 10% as drop-out, total sample equals 105 patients.

Specimen collection and processing

Urine, tracheal aspirates, throat swabs, ear swabs, blood, high vaginal swabs, wound swab, skin samples, and nail specimens were collected under aseptic precautions and processed in Microbiology Lab, Faculty of Medicine, Suez Canal University for isolation and identification of *Candida* species. Specimen processing included direct smearing and microscopical examination using wet mount preparations, KOH preparation and Gram staining. *Candida* isolation was done on Sabouraud dextrose agar (SDA) (Biolab, Hungary) supplemented with chloramphenicol (0.5 gm/L) and gentamicin (5 mg/L). *Candida* species were phenotypically identified using germ tube test, the ability to grow in hypertonic Sabouraud broth, microscopic morphology on corn meal agar (Condalab, Spain), growth and coloration on chromogenic *Candida* agar (TMmedia, India) and biochemical reactions testing using KB006 Hi*Candida* Identification Kit (Himedia, India) and Vitek 2 YST-ID system (BioMérieux, France) [8].

Antibiotic susceptibility testing

Antifungal susceptibility testing of the isolated *Candida* species was done by disk diffusion method on Muller-Hinton agar (Himedia, India) supplemented with 2% glucose and 0.5 μg/mL of methylene blue. The used antifungal disks were fluconazole (25 μg), voriconazole (1 μg) and amphotericin B (100 μg). Inhibition zones were

interpreted according to the CLSI interpretive breakpoints for fluconazole and voriconazole. While for amphotericin B, the interpretive breakpoints were implemented from previously published studies [9-10].

Quality control

Reference strain *C. albicans* American Type Culture Collection (ATCC) 10231 (Miclev, Sweden) was used as a control strain.

Statistical analysis

Statistical analysis was performed using SPSS-25 software (SPSS Inc., Chicago, IL, USA). Data was analyzed and presented as numbers and percentages using tables and graphs with the confidence interval (CI) at 95%. *p* value of 0.05 was used as the limit of statistical significance.

Results

This study included 105 clinical specimens collected from clinically suspected cases of *candida* infection in different departments in SCUHs. Twenty-nine specimens were collected from patients in outpatients' clinics while 76 specimens were collected from patients admitted at inpatient departments. Out of the 105 collected specimens, 57 (54.3%) showed positive *Candida* growth. *C. tropicalis* was the most common isolated species 19(18.1%) followed by *C. albicans* 14 (13.3%), *C. dubliniensis* 11(10.5%), *C. glabrata* 7(6.7%), *C. parapsilosis* 5(4.8%) and lastly *C. kefyr* 1(1%) **(Figure 1).**

Candida albicans and *C. dubliniensis* were indistinguishable by chromogenic agar as both species produced light green color on this medium **(Figure 2).**

Final species identification was made based on the agreement of the results between the different methods for the same species **(Table 1).**

Reference strain *C. albicans* ATCC 10231 was correctly identified by corn meal agar, chromogenic agar and Vitek 2 YST-ID system. However, KB006 Hi*Candida* identification kit could not identify it.

Of the 57 isolated strains, 56(98.25%) strains were susceptible to amphotericin B and 1(1.75%) was resistant. Forty-seven (82.5%) strains were susceptible to fluconazole and 2(3.5%) were resistant. Fifty (87.7%) strains were susceptible to voriconazole, and 7(12.3%) isolates were *C. glabrata*. For *C. glabrata* and voriconazole, current data are insufficient to demonstrate a correlation between *in vitro* susceptibility testing and clinical outcome **(Table 2).**

Among the isolated species, only one isolate of *C. tropicalis* showed resistance to amphotericin B with statistical insignificant differences as *p*=1.00. One isolate of *C. albicans* and *C. parapsilosis,* and 6 isolates of *C. glabrata* were susceptible dose dependent to fluconazole with statistically significant differences as *p*<0.001 **(Table 3).**

Table 1. Identified species by different identification methods.

Identified species	Corn meal agar	Chromogenic agar	KB006 Hi*Candida* identification kit	Vitek 2 YST-ID system	Final species identification
C. albicans	13	25	3	4	14
C. dubliniensis	12			1	11
C. parapsilosis	1	0	2	5	5
C. glabrata	7	13	1	0	7
C. kefyr	2	0	3	1	1
C. tropicalis	16	18	5	7	19
C. lusitaniae	1	0		1	0
C. krusei	0	1	0	0	0
Not recognized	5	0	5	0	0
Not used	0	0	38	38	0
Total	**57**	**57**	**57**	**57**	**57**

Table 2. Antifungal susceptibility patterns for isolated strains using disc diffusion method.

Antifungal drug	Susceptibility patterns			Total
	Susceptible	**Susceptible dose dependent**	**Resistant**	
Amphotericin B	56(98.25%)	0(0%)	1(1.75%)	57
Fluconazole	47(82.5%)	8(14%)	2(3.5%)	57
Voriconazole*	50(87.7%)	0(0%)	0(0%)	50

* For *C. glabrata* (7 isolates) and voriconazole, current data are insufficient to demonstrate a correlation between *in vitro* susceptibility testing and clinical outcome.

Table 3. Antifungal susceptibility patterns for the isolated species using disc diffusion method.

Antifungal drug	Susceptibility patterns	*C. albicans*	*C. dubliniensis*	*C. tropicalis*	*C.glabrata***	*C. parapsilosis*	*C. Pseudo tropicalis*	*p*-value
AmB	S	14	11	18	7	5	1	1.00
	R	0	0	1	0	0	0	
	T	14	11	19	7	5	1	**57**
FLZ	S	13	11	19	0	3	1	
	SDD	1	0	0	6	1	0	**<0.001***
	R	0	0	0	1	1	0	
	T	14	11	19	7	5	1	**57**
VSZ	S	14	11	19	0	5	1	
	R	0	0	0	0	0	0	
	T	14	11	19	0	5	1	**50**

The test used Chi-square test or Fisher exact test.

Abbreviations: AmB = Amphotericin B, FLZ= Fluconazole, VSZ = Voriconazole, S=susceptible, R= resistant, SDD=susceptible dose dependent., T= total.

*Statistically significant as p<0.05.

** For *C. glabrata* and voriconazole, current data are insufficient to demonstrate a correlation between *in vitro* susceptibility testing and clinical outcome.
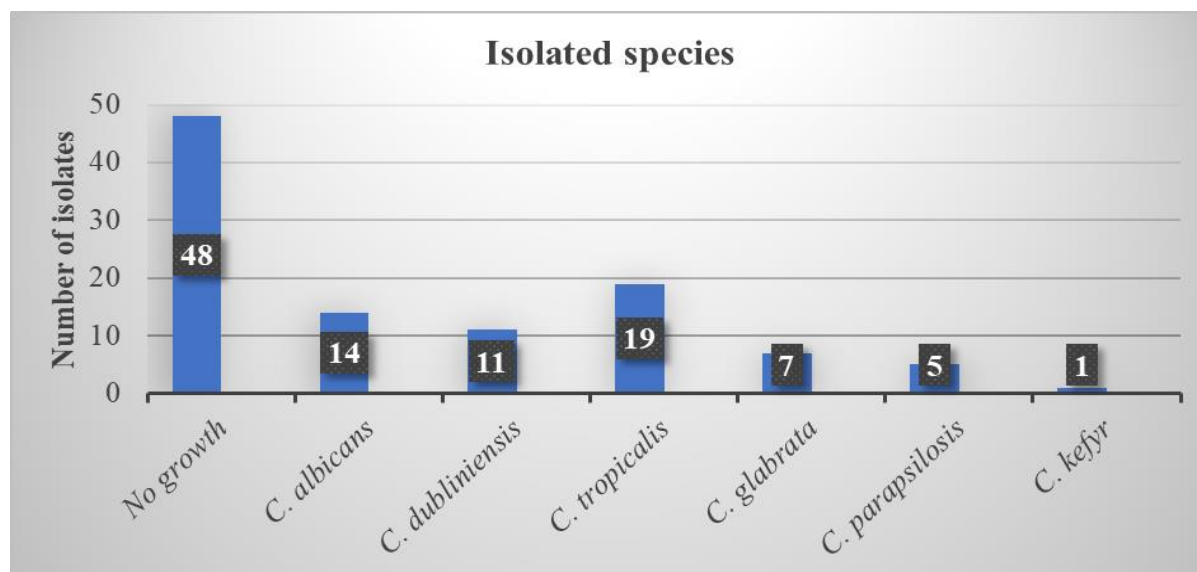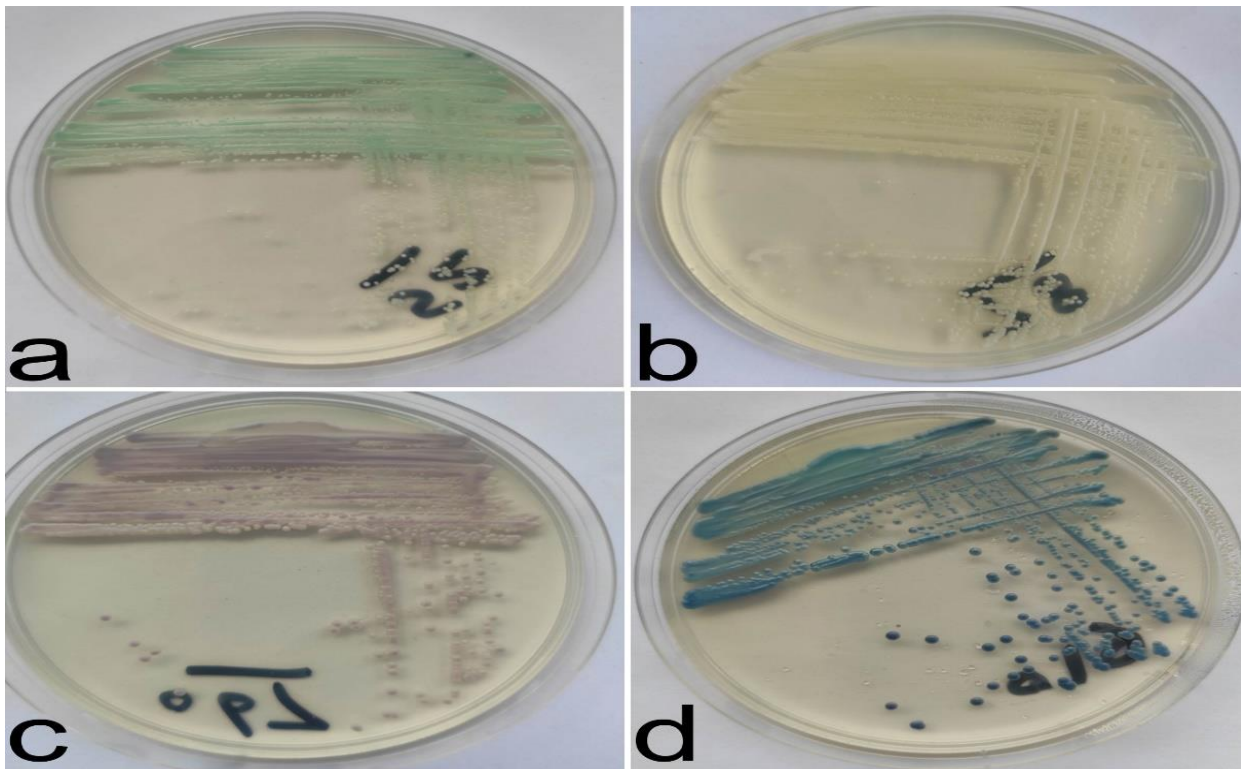
Figure 1. Isolated species.

Figure 2. Color of different species on chromogenic agar.

a: *C. albicans* or *C. dubliniensis*, b: *C. glabrata*, c: *C. krusei*, d: *C. tropicalis*.

Discussion

Candida is the most common cause of fungal infections worldwide. identification of *Candida* species is important for better selection of antifungal agents [2].

Numerous antifungal classes are used to treat *Candida* infections. However, resistance among previously susceptible *Candida* species has emerged following the expanded use of the antifungal drugs [4].

The current study aimed to improve the diagnosis and prognosis of candidiasis through identifying the different *Candida* species isolated from various clinical specimens in SCUHs, using different phenotypic methods and to assess their antifungal susceptibility patterns.

In the current study, 105 clinical specimens were collected from clinically suspected cases of candidiasis in different departments of SCUHs.

Out of the 105 specimens, 57 (54.3%) showed positive *Candida* growth. *C. albicans* accounted for 24.6% of the isolated species while non-*albicans Candida* species (NACs) were the most prevalent (75.4%). This finding is comparable to the NAC prevalence of 76% reported by **El-sayed**

and **El-khayat** [11], and the 75% reported by **Daef et al.** [12]. These findings support the worldwide shift of *Candida* infections in the last few decades towards NAC species. However, **Kadry et al.** [13] and **El-Ganiny et al.** [14] had reported lower prevalence of NACs of 42.6% and 29.3%, respectively.

Among the isolated species, *C. tropicalis* was the most prevalent (33.3%) followed by *C. albicans* (24.6%). Similar results obtained from **Daef et al.** [12] study in which *C. tropicalis* was the most frequently isolated species (46.5%) followed by *C. albicans* (25%).

El-sayed and El-khayat [11] study reported *C. albicans* as the most frequently isolated species responsible for fungal urinary tract infections with a prevalence of 45.5%. **Emam et al.** [15] and **Mokhtar** [16] also reported *C. albicans* as the most common species responsible for vulvovaginal candidiasis. Moreover, *C. albicans* was the most prevalent in **El-Ganiny et al.** [14] study (57.4%), while the prevalence of *C. tropicalis* was 6.5%. **Abass et al.** [17] and **Khairat et al.** [18] had reported lower prevalence rates for *C. tropicalis* (16% & 11%, respectively) in candidemia cases.

In the present study, *C. dubliniensis* had a prevalence of 19.3%. Variable prevalence rates of *C.*

dubliniensis were detected in different clinical specimens and different countries all over the world. As, 9% was reported in Germany, 11% in Sweden and 4.9% in Egypt [14,19,20].

The present study revealed a prevalence of *C. glabrata* of 12.3%. **Yang et al.** [21] revealed a similar prevalence of 13-20% for *C. glabrata* in different clinical specimens, while **Sandhya et al.** [22] reported a *C. glabrata* prevalence of 5.2% in diabetic patients. Moreover, it had a prevalence rate of 4.9% in **El-Ganiny et al.** [14] study.

Candida parapsilosis had a prevalence of 8.8% in the current study. **Sandhya et al.** [22] and **El-Ganiny et al.** [14] had comparable *C. parapsilosis* prevalences of 6.1% and 5.7%, respectively. However, higher *C. parapsilosis* prevalences (20% and 21%) had been reported in cases of neonatal invasive candidiasis in England and Canada, respectively [23,24].

Candida kefyr had the lowest prevalence (1.8%) in the current study. Similar results were obtained in a study performed in Kuwait which reported an overall prevalence of 0.83% for *C. kefyr* in different types of candidiasis and a prevalence of 0.32% for *C. kefyr* in cases of candidemia [25]. However, in a study of candidemia in patients with hematological malignancies, *C. kefyr* was responsible for about 10% of cases [26].

Variations in different species prevalence are related to many factors including the geography of the study, the type and quality of collected specimens, the number of collected specimens, the characterizations of the study population and the study methodology. In addition to factors that affect candidiasis distribution like nosocomial infection rates, source of infection and antifungal drug therapy.

Among all the used methods in this study, Vitek 2 YST-ID system had the most accurate results. Thus, it can be used alone for identification of *Candida* species. In cases of shortage of resources, combination between chromogenic media and corn meal tween 80 agar can be useful in identifying most of the medically important *Candida* species.

Regarding antifungal susceptibility, disc diffusion method was used in the current study. Resistance was observed against fluconazole (3.5%) and amphotericin B (1.75%). The resistance was detected only in NACs. The species that showed fluconazole resistance were *C. glabrata* and *C. parapsilosis*. The species that showed amphotericin B resistance was *C. tropicalis*. Moreover, eight (14%) susceptible dose dependent strains to fluconazole were detected. These strains were 6 (10.5%) *C. glabrata*, 1 (1.75%) *C. albicans* and 1(1.75%) *C. parapsilosis*. No resistance to voriconazole was detected in the current study.

The results regarding *C. glabrata* were consistent with **Vermitsky and Edlind** [27] study that reported *C. glabrata* to be intrinsically less susceptible to azoles and able to develop acquired resistance during treatment.

Bhattacharjee [28] reported higher resistance rates to fluconazole which was 34.38%. **Khairat et al.** [18], **Ghonaim et al.** [29] and **El-Ganiny et al.** [14] also reported higher levels of resistance against fluconazole which were 41.8%, 33.3% and 13.1%, respectively. However, lower fluconazole resistance rates were detected in Taiwan, Egypt, Brazil, and Portugal [10, 30- 32]. The lowest fluconazole resistance rates (0.6%) were reported in Australia and Kuwait [33,34].

These variations in antifungal susceptibility profiles may be due to differences in the sources and types of the clinical specimens, differences of *Candida* species distribution and antifungal drugs usage in the different geographical areas.

Being a study for fulfilment of master's degree in a regional university hospital, the present study has its limitations of small sample size, absence of funds, limited experience of the researcher, as well as abuse of azoles -especially fluconazole- as empirical therapy for cancer patients at the oncology and nuclear therapy department in the hospital. Thus, they were excluded from the study population. However, the study proved that *C. albicans* is no longer the most commonly isolated species as NACs are becoming more prevalent. In addition, chromogenic *Candida* agar and corn meal agar are valuable methods for identification of *Candida* species even in poor resource settings. Despite of the extensive use of antifungal drugs, antifungal resistance rates among *Candida* spp. are still low.

Conclusion

This study revealed that the prevalence of candidiasis is remarkable. Moreover, NACs cause most of these infections which change the concept of *C. albicans* predominance. Despite the extensive use of antifungals, fluconazole and amphotericin B

showed low resistance rates. No resistance to voriconazole was reported. Therefore, voriconazole could be more effective as empirical therapy than fluconazole and amphotericin B. Finally, accurate identification of *Candida* species is essential for therapeutic and prognostic impact, appropriate selection of antifungal agents by clinicians and controlling the increase of resistant *Candida* strains.

Conflicts of interest

The authors declare that they have no financial or non-financial conflicts of interest.

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