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ORIGINAL ARTICLE

Automated Identification and Antifungal Susceptibility Testing of *Candida* Species using Vitek 2 Compact System in ICUs and Pediatric Oncology Unit, Alexandria, Egypt

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ABSTRACT

Background: *The epidemiology of candida species has changed over the last two decades. Early species identification and rapid antifungal susceptibility testing (AFST) is mandatory in such critical patients.* ***Objectives:*** *To determine the prevalence of different types of Candida species in ICU and pediatric oncology patients as well as their antifungal susceptibility profile using the Vitek 2 compact system.* ***Methodology:*** *1023 candida isolates were collected from different clinical samples between May 2014 and February 2017. Identification and antifungal susceptibility testing were performed using Vitek 2 compact system.* ***Results:*** *In the present study fifteen candida species were identified. Non albicans Candida species (NAC) were predominant (65.6%). Candida albicans was the most prevalent species (34.5%). Candida tropicalis (c.tropicalis) was the most common isolated NAC species and the second common isolated species (32.8%), followed by Candida glabrata (C.glabrata) (17.3%), Candida Parapsilosis (C.parapsilosis)(7.5%), accounting for 92.1% of the total number of isolates.Uncommon Candida species were also isolated in this study.C. tropicalis was the most frequent species isolated from urine (50.9%), while C. albicans was the most frequent species isolated (43.3%) & (30.8%) from respiratory and blood samplesrespectively.C. parapsilosis was the most common species isolated from indwelling devices (44.4%). Regarding antifungal susceptibility profile (5.1%) and (2 %) of C.albicans were resistant to fluconazole, and voriconazole repectively. C. tropicalis (24.6%) and (17.5%) were resistant to Fluconazole and voriconazole respectively. (13.9%), (7.2%), (2.4%) of C.glabrata isolates were resistant to fluconazole, voriconazole and amphotericin respectively. All C. parapsilosis isolates were sensitive to fluconazole, voriconazole. Although resistant to fluconazole, all C. krusei isolates were susceptible to voriconazole, while, (84.6%) of isolates were resistant to flucytosine.* ***Conclusions:*** *The increased isolation rates of NAC species and gradual shift in the antifungal susceptibility profile underlines the need of early and accurate diagnosis of infecting Candidaspecies along antifungal susceptibility testing.*

Keywords:

INTRODUCTION

The incidence of invasive fungal infections have increased since the 1980s, especially in immunocompromised patients and those hospitalized with serious underlying diseases.[1]

Several factors have contributed to the rise of invasive Candida infections in recent decades including immunosuppressive therapy, chemotherapy, increasing utilization of implantable devices, as well as increased use of antibiotics.[2, 3]

Although the distribution of Candidaspecies varies across geographic regions, Candida *albican*sis by far the predominant species of candidiasis.[4] However,a shift towards NAC species hasbeen rising.Species commonly isolated include C.*tropicalis*, C.*parapsilosis*, C.*glabrata*, C.*guilliermondii*, C.*dubliniensis*, and C.*krusei*.[5, 6]

C. *tropicalis* is one of the most common Candida species that causes disease in humans, especially in tropical climates [7,8].C. *parapsilosis* has emerged as a significant nosocomialpathogen usually associated with invasive procedures orprosthetic devices [9]C.*glabrata*is the second most common species after C. albicans in North America.[10]

Considered that the outcome of invasive fungal infections, in particular, candidemia, is improved by early initiation of appropriate antifungal therapy [11] and given that many NACspecies are resistant or less susceptible to antifungal agents, therefore the need to determine the antifungal susceptibility patterns of candida isolates has become of great importance.[12-14]

The VITEK 2 system (BioMurieux, Durham, US) is a fully automated system designed for the identification and susceptibility testing of candida species. It allows the identification of clinically important yeasts and yeast-like organisms due to a sensitive fluorescence-based technology. The ID-YST database of VITEK 2 for yeast identification comprises 51 different taxa, including newly described species, taking into account recent advances in taxonomy.[15]

Nonetheless, data concerning the profile and antifungal susceptibility of *Candida* spp. are relatively few in some Arab countries and other countries in the region.[16] Knowledge of recent local epidemiological patterns, and susceptibility to antifungals is of great importance.[6]

The purpose of the present work is to describe the current epidemiology and antifungal susceptibility profile of *Candida* species isolated from different anatomical sites from ICUs and pediatric oncology patients in Alexandria, Egypt.

METHODOLOGY

A total of 1023 *candida* isolates were collected from different clinical samples between May 2014 and February 2017. Samples were collected from various ICUs in Alexandria and from pediatric oncology unit in Alexandria university hospital, Egypt.

Recurrent strains obtained from the same materials in the same patient and isolates unidentified as to species were not included in the study.

All *Candida* isolates were initially sub-cultured onto Sabouraud dextrose agar (SDA) medium and incubated at 35°C till growth appeared.

Inoculum suspension.

The inoculum suspensions for the VITEK 2 were prepared in sterile saline at a turbidity equal to a 2.0 McFarland standard, as measured using a DensiChek instrument (bioMérieux). The individual test cards were automatically filled with the prepared culture suspension, sealed, and incubated by the VITEK 2 instrument.

The identification YST cards were incubated at 35.5 °C for 18 hours, and optical density readings were taken automatically every 15 minutes. Each profile was interpreted according to a specific algorithm and the final profile results were compared with the ID-YST database, which led to the final identification of the microorganism.A final identification of "excellent," "very good," "good," "acceptable," was considered to be correct.

Antifungal susceptibilities of all isolates were determined using the Vitek 2 system (AST-YS07 card, bioMérieux, Marcy L'Etoile, France) according to the manufacturer's instructions. Quality control was performed by testing *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

The VITEK 2 cards containing serial two-fold dilutions of amphotericin B, caspofungin, fluconazole, flucytosine, micafungin and voriconazole were provided by the manufacturer. The time of incubation varied from 10 to 30 hours based on the growth rate in the drug-free control well, and the results were expressed as minimal inhibitory concentration (MICs) in micrograms per milliliter.

The antifungal MICs were determined based on the new, recently approved, Clinical and Laboratory Standards Institute (CLSI) species-specific clinical breakpoints (SS-CBPs) (CLSI M27-S4) as well as European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints in March 2013.[17, 18]

The new CLSI breakpoint2012 were applied for caspofungin. Epidemiological cut-off values (ECVs) were applied for flucytosine and amphotericin B for C. *tropicalis* and were also used for voriconazole against C. *glabrata*as no breakpoint was defined by CLSI 2012 and EUCAST due to insufficient evidence.[19-21]

Table 1: Antifungal clinical breakpoints (in mg/l)

Fluconazole					
	New CLSI bp (2012)			**EUCAST bp (2013)**	
Candida species	**S**	**SDD**	**R**	**S**	**R**
C. albicans	≤ 2	4	≥ 8	≤ 2	> 4
C. tropicalis	≤ 2	4	≥ 8	≤ 2	> 4
C. parapsilosis	≤ 2	4	≥ 8	≤ 2	> 4
C. glabrata		≤ 32	≥ 64	≤ 0.002	> 32
C. krusei	R	R	R	-	-
Voriconazole					
	New CLSI bp (2012)			**EUCASTbp(2013)**	
***Candida* species**	**S**	**I**	**R**	**S**	**R**
C. albicans	≤ 0.12	0.25 – 0.50	≥ 1	≤ 0.12	> 0.12
C. tropicalis	≤ 0.12	0.25 – 0.50	≥ 1	≤ 0.12	> 0.12
C. parapsilosis	≤ 0.12	0.25 – 0.50	≥ 1	≤ 0.12	> 0.12
C. glabrata	IE	IE	IE	IE	IE
C. krusei	≤ 0.5	1	≥ 2	IE	IE
Caspofungin					
	New CLSI bp (2012)				
***Candida* species**	**S**	**I**	**R**		
C. albicans	≤ 0.25	0.5	≥ 1		
C. tropicali	≤ 0.25	0.5	≥ 1		
C. parapsilosis	≤ 2	4	≥ 8		
C. glabrata	≤ 0.125	0.25	≥ 0.5		
C. krusei	≤ 0.25	0.5	≥ 1		

bp, breakpoint; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; I, intermediate; IE, insufficient evidence; R, resistant; SDD, dose-dependent susceptible; S, susceptible; –, antifungal susceptibility testing not recommended.

Candida isolate is considered susceptible when its MIC was at or below the breakpoint defined by EUCAST or CLSI. Non-susceptible or resistant isolate is considered when its MIC was higher than the breakpoints defined by EUCAST/CLSI and includes both dose-dependent susceptible, intermediate and resistant isolates.

RESULTS

Samples distribution:

The study included 1203 samples identified by the Vitek 2compact system. Samples included: (38.2%) urine, (29.3%) Respiratory samples (Sputum, BAL, MiniBAL and E.T.T), (19.7%) blood, (11.1%) swabs and aspirates from surgical site lesions. (0.7%) indwelling devices, (0.7%) from intraperitoneal fluid and (0.2%) from C.S.F

Species distribution:

NAC species were predominant over C.*albicans* species (788; 65.6%), however Candida *albicans* was the most prevalent species (415; 34.5%). C.*tropicalis*was the most common isolated NAC species and the second common isolated species (394; 32.8%), followed by *C.glabrata* (208; 17.3%), *C. Parapsilosis* (90; 7.5%), accounting for 92.1% of thetotal number of isolates. The remaining 7.9% of the speciesincluded *C. krusei* (39; 3.2%), *C. kefyr* (11; 0.9 %), *C. guilliermondii* (11; 0.9%), C. *lusitaniae* (10; 0.8%), *C.dubliniensis* (8; 0.7%), *C.lipolytica*(6; 0.5%). *C. famata* (4; 0.3%), *C. rugosa* (3; 0.2%), *C. pelliculosa* (2; 0.2%), C. norvegensis (1; 0.1%), and C. *spherica* (1; 0.1%)

In the current study, *C. tropicalis* was the most frequent species isolated from urine (50.9%) followd by *C. albicans* (26.3%) and *C.glabrata* (15.7%), while in respiratory samples*C. albicans* was the most frequent species isolated (43.3%) followd by *C. glabrata* (28%) and *C.tropicalis* (18.7%). In candidemia patients *C. albicans* was the most frequent species isolated from blood (30.8%) followed by *C. parapsilosis* (28.7%). Also *C. parapsilosis* was the most common species isolated from indwelling devices (44.4%) followed by *C.tropicalis* (33.3%).

Table 2: Distribution of candida species according to specimen sources

specimen sources		Candida species														
		C. albicans	C. tropicalis	C. glabrata	C. krusei	C. parapsilosis	C. famata	C. spherica	C. pellculosa	C. lusitaniae	C. guilliermondii	C. dubliniensis	C. kefyr	C. rugosa	C. lipolytica	C. norvegensis
Urine (n = 460)	No.	121	234	72	12	5	2	0	0	6	1	0	1	1	4	1
	%	26.3	50.9	15.7	2.6	1.1	0.4	0.0	0.0	1.3	0.2	0.0	0.2	0.2	0.9	0.2
Blood (n = 237)	No.	73	55	16	5	68	1	1	2	3	7	2	1	1	2	0
	%	30.8	23.2	6.8	2.1	28.7	0.4	0.4	0.8	1.3	3.0	0.8	0.4	0.4	0.8	0.0
Respiratory (n = 353)	No.	153	66	99	18	1	0	0	0	1	1	4	9	1	0	0
	%	43.3	18.7	28.0	5.1	0.3	0.0	0.0	0.0	0.3	0.3	1.1	2.5	0.3	0.0	0.0
Surgical site lesion (n = 133)	No.	66	34	15	3	11	1	0	0	0	2	1	0	0	0	0
	%	49.6	25.6	11.3	2.3	8.3	0.8	0.0	0.0	0.0	1.5	0.8	0.0	0.0	0.0	0.0
Intra peritoneal Fluid (n = 9)	No.	1	1	6	1	0	0	0	0	0	0	0	0	0	0	0
	%	11.1	11.1	66.7	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
In dwelling Device (n = 9)	No.	1	3	0	0	4	0	0	0	0	0	1	0	0	0	0
	%	11.1	33.3	0.0	0.0	44.4	0.0	0.0	0.0	0.0	0.0	11.1	0.0	0.0	0.0	0.0
C.S.F (n = 2)	No.	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
	%	0.0	50.0	0.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Qualitative data were described using number and percent

Antifungal susceptibility profile:

In the current study the antifungal susceptibility testing of only 5 species of *candida* (*C.albicans, C.tropicalis, C. glabrata, C. krusei,and C. parapsilosis*) could be determined and interpretreted by Vitek 2 compact system using breakpoints defined by EUCAST, CLSI as well as epidemiological cut-off values.

As regards *C.albicans,* the majority of isolates were sensitive to azoles whereas only (5.1%) and (2%) were resistant to fluconazole and voriconazole respectively. while all isolates were sensitive to caspofungin and micafungin. (Fig 1)

C.*tropicalis:* (24.6%) were resistant to Fluconazole, (17.5%) were resistant to voriconazole. All isolates were sensitive to Amphotericin, caspofungin and micafungin, while only (1%) were resistant to Flucytosine. (Fig 2)

C.glabrata: Majority of isolates (86.1%), (92.8%), (97.6%) were sensitive to fluconazole, voriconazole and amphotericin respectively. All isolates were sensitive to caspofungin and micafungin whereas only (0.5%) were resistant Flucytosine. (Fig 3)

C. krusei: All isolates were fluconazole resistant but sensitive to voriconazole, majority of isolates (84.6%) were resistant to Flucytosine. (Fig 4)

C.parapsilosis: All isolates were sensitive to fluconazole, voriconazole, caspofungin, micafungin and flucytosine, while only (3.3%) were resistant amphotericin (Fig 5)

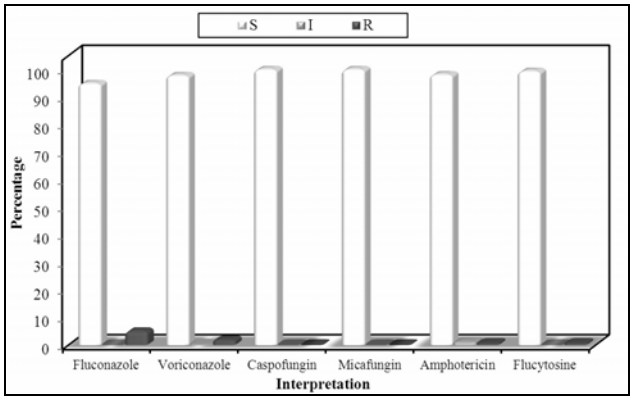

Fig. 1: Antifungal susceptibility profile of *C.albicans*

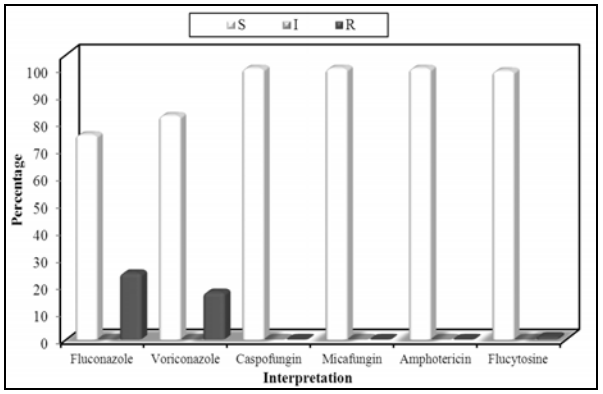

Fig. 2: Antifungal susceptibility profile of *C. tropicalis*

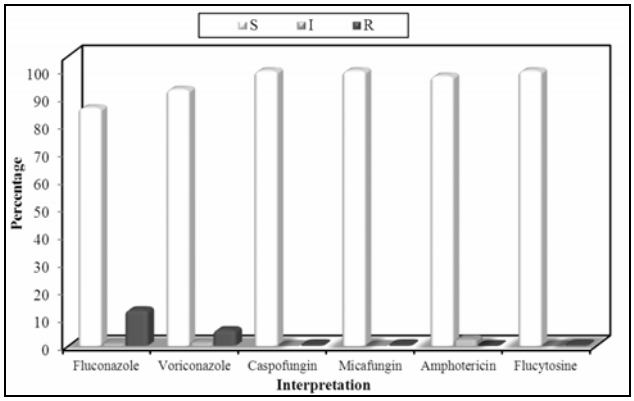

Figure (3): Antifungal susceptibility profile of *C.glabrata*

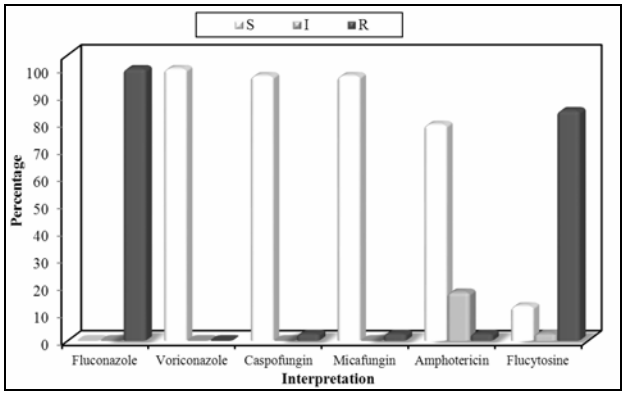

Fig. 4: Antifungal susceptibility profile of *C.kruzei*

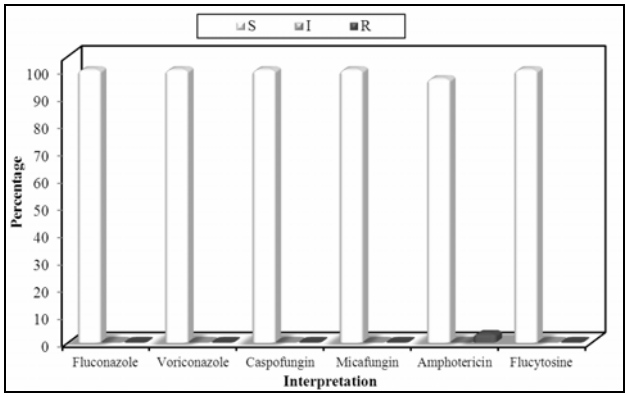

Fig. 5: Antifungal susceptibility profile of *C.parapsilosis*

DISCUSSION

The epidemiology of fungal infections has become an important issue in the past two decades. *Candida* species represent the most frequently isolated fungal pathogens and are leading causes of fungal infections worldwide. During last 20 years, an epidemiological shift has been observed among *Candida* species in patients with candidiasis, the incidence of *C. albicans* has decreased, while that of the NAC has been rising.[6]

In Egypt, data concerning candidiasis epidemiology are lacking. To our knowledge, this study could be reported as the first large-scale surveillance study of the distribution of Candida species in Egypt. This study showed a remarkable diversity of *Candida* species (15 species) isolated from clinical samples. In this study, NAC species were predominant (65.6%) denoting a significant change in candidiasis epidemiology from albicans to NAC

Our finding was in agreement with several studies. In a large study conducted by *Horn et al*. with 2019 patients at major North American medical centres, a predominance of NAC was observed. [22].

Ajenjo H et al[23]also observed Changes in candidiasis epidemiology in Latin American countries, with progressive increase in NAC infection where*C. parapsilosis* was the most frequent species, followed by *C. tropicalis* and *C. glabrata*. Another two studies by *Shivanand et al and Kashid et al*[24, 25]also showed the NAC incidence to be higher than that of *C. albicans.*

C. tropicalis ranks between the second or third NAC most frequently isolated from patients with *Candida* infections. In the present study, *C.tropicalis* surpassed the other NAC to become the most common species isolated second to *C.albicans*. A 9-year long study conducted by *Yap et al.*[26], where NAC accounted for 46% of isolates collected in Hong Kong, *C. tropicalis* was the most common NAC and was also the most common NAC amongthe *Candida* bloodstream isolates studied in many studies in different regions of Asia.[27.28]

Several studies have shown that *C. glabrata* has emerged as an important causative agent of candidiasis [29, 30].Previous epidemiological studies displayed a mycological shift from predominately *C. albicans* to *C. glabrata*[31 32]. *C. glabrata* was the most common of the NAC species, accounting for 49.4% of NAC infections in Prospective Antifungal Therapy Alliance (PATH Alliance) registry from 2004 to 2008 including a total of 2,496 patients with NAC species [33]. This finding were in contrast to our finding where *C. glabrata* in our study was the third common Candida species isolated, which does not indicate a shift towards *C. glabrata* as an emerging causative agent of candidiasis in ICUs units in Egypt.

Pfaller et al study reported *C. Parapsilosis* to be the second most common frequently isolated NAC species in their study [33] while in a study conducted in Chinese hospitals [34]the most common NAC species encountered was *C. parapsilosis* (36.6%), followed closely by *C. tropicalis* (35.4%).These results were contradictory to our findings; as in our study *C. parapsilosis* ranked the fourth common isolated candida species representing only (7.5%) of isolates.

Our results were also different from findings in a large prospective, laboratory-based, multicentre study of invasive yeast infections in china including A total of 1072 isolates; C. *parapsilosis* was the most common

isolated species (36.6%) followed by (35.4%) *C. tropicalis*, (24.3%), *C. glabrata* and (3.7%) *C. krusei*.[35]

In the present study *C. krusei*, was fourth in rank order among NAC species isolated. Similar finding was also reported by several other studies in which *C. Krusei* was the fourth common isolated NAC. [33-36]

NAC species were recovered from all samples included in this study. In present study *C. tropicali s*was the predominant species (50.9%) isolated from urine culture, while *C. parapsilosis* was the predominant isolate (44.4%) from indwelling devices and the second common species isolated from blood cultures (28.7%). *C.albicans* was the most common species isolated from both respiratory specimens (43.3) and blood cultures 30.8%).

In a large prospective laboratory-based surveillance study[37]of *Candida* species conducted in Malaysia on 34 392 clinical samples, *C. albicans* was the predominant species isolated from the respiratory tract while the Candida species isolated from blood, urine and pus were predominant NAC.*C. tropicalis* was the most frequent species isolated from urine (59.03%).

The changing epidemiology of NAC infections has generated concern about the emergence of antifungal resistance and their relevance to clinical outcome. A reduced antifungal susceptibility in NAC and a correlation with routine fluconazole prophylactic use has been suggested.[38]

In the current study the demonstrated antifungal resistance was mainly related to azoles as (24.6%) & (17.5%) of C.tropicalis isolates were resistant to fluconazole, and voriconazole respectively compared to (13.0%) & (5.8%) of *C.glabrata* isolates, *while C.albicans*, only (5.1%) were resistant to fluconazole and (2 %) were resistant to voriconazole.

Our results were in agreement with *Pu et al* study [34] who reported in their study thatpatients infected with *C. albicans, C. tropicalis, and C. parapsilosis* had a high susceptibility rate to the antifungal agents (amphotericin B, flucytosine, fluconazole, and voriconazole) (>90%). These were consistent findings with some previous reports, which suggested that azole resistance was uncommon in *C. albicans, C. parapsilosis, and C. tropicalis* (<10%).[39-41]

However, Our results are in contrast to several other studies[39-42] which reported higher rates of resistance to fluconazole in *C. albicans* strains 26.4%, 45.83%, and 74.2% respectively.

Higher percentage of azole resistance was reported by *Baghdadi et al* study [36] in which 41.6% of *C. tropicalis* isolates were resistant to both fluconazole, and voriconazole. Low percentage of azole resistance was reported in china [35] as 11.6% and 9.5% of *C. tropicalis* isolates were non-susceptible to fluconazole and voriconazole, respectively.

In the present study no resistance to fluconazole and voriconazole was demonstrated among *C. parapsilosis*. Similarly *Shokohi et al*. [39]found no azole resistance among their isolates however, *Zhang et al*.[43] findings showed (15.4%) resistance to fluconazole.

In the present study (13.9%), (7.2%), (2.4%) of *C.glabrata* isolates were resistant to fluconazole, voriconazole and amphotericin respectively. All isolates were sensitive to caspofungin and micafungin whereas only (0.5%) were resistant Flucytosine. Similar findings were reported in china [35] where 14.3% of C. glabrata isolates were fluconazole resistant. An overall, 97.7%–100% of isolates were susceptible to caspofungin and micafungin

Our findings were incontrast to the shift towards more resistant *C.glabrata* species described in several European countries and in the USA [44].An increase of non-susceptibile isolates was observed among C. glabrata for azoles in the Swiss study[45] reported (39.8%) of isolates to benon-susceptible to fluconazole.

Although resistant to fluconazole, all C. krusei isolated in this study were susceptible to voriconazole. Different results were reported by 6-year prospective candidaemia survey from the fungal infection network of Switzerland which stated that (18.2%) C. krusei isolates tested were non- susceptible to voriconazole.

The high prevalence of NCAC species in disease could also be a reflection of their inherently higher level of resistance to certain antifungal drugs [46] compared with C. albicans, as this would promote their persistence, possibly to the detriment of C. albicans, in mixed species infections treated with traditional antifungal agents.

CONCLUSIONS

In conclusion, this study has provided important data on increased isolation rates of NAC and antifungal susceptibility of common NAC species in Egypt. Four species *(C. albicans, C. tropicalis, C. glabrata and C. parapsilosis)* represented more than 90% of isolates, *C. albicans* remained the predominant species while C. tropicalis species was the most common NAC species. The study also demonstrated reduced susceptibility among C. tropicalis for the azoles. Such findings emphasize the need to perform more locally relevant epidemiological studies, as well as studies to correlate *in vitro* resistance with clinical outcomes.

We believe that the data obtained from this study could help in developing clinical practice recommendations to improve the management of invasive candidiasis.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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