

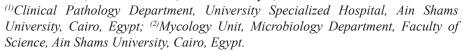
### **Egyptian Journal of Microbiology**

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## Incidence of ICU-Acquired Candidemia in a Tertiary Care Hospital in Cairo, Egypt

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THIS LABORATORY-BASED study aims to report ICU-acquired candidemia incidence rate and the circulating *Candida* spp. in a university tertiary care hospital for the first time in Egypt. This study was conducted between January 2013 to December 2017 at the Ain Shams University Specialized Hospital located in Cairo. The incidence rate and species distribution as well as antifungal susceptibility testing of isolated Candida spp. against fluconazole, voriconazole, caspofungin, and micafungin was determined. During the study period, a total of 50 proven candidemia episodes were recorded. The incidence rate is 3.3 cases/1,000 ICU admission-year amounting to a burden of 33.33 cases/100,000 inhabitants-year (95% CI 32.97 - 33.70). Out of the 50 isolated *Candida* species, *C. albicans* accounted for 26% while Nonalbicans species accounted for 74%. The rank order of Candida spp. was C. krusei 14 (28%), C. albicans 13 (26%), C. parapsilosis 10 (20%), C. tropicalis 8 (16%), and C. glabrata 5 (10%). The antifungal susceptibility testing revealed that all isolated Candida spp. exhibited variable resistance rates to the four tested antifungal agents where low rates of resistant isolates were observed for caspofungin (24%) while the high rates were observed for fluconazole (50%). The results obtained indicated that the incidence rate of candidemia and the emergence ratio of Nonalbicans Candida spp. is the highest compared to neighboring countries in the Middle East.

 $\textbf{Keywords:} \ \textbf{Candidemia}, \ \textbf{ICU}, \ \textbf{Incidence}, \ \textbf{Candida} \ \textbf{spp.} \ \textbf{distribution}, \ \textbf{Egypt} \ .$ 

#### Introduction

ICU-acquired candidemia is the *Candida* bloodstream infection (CBSI) which diagnosed after more than 48 h ICU admission and considered proven at the time of first positive blood culture. The major risk groups are the hematological malignancy and critically ill patients (Antinori et al., 2016).

Candidemia is a life-threatening infection that represents the highest incidence of fungal infections in developed and developing countries with mortality rates ranging from 25-60%. The inability to manage the disease in developing countries increases hospital stay and raises the cost of treatment, which increases disease burden. Although many diagnostic and

therapeutic tools have been used for candidemia management in developed countries, empirical therapy is the adopted way in most of developing countries (Kaur & Chakrabarti, 2017).

In Egypt, the health care settings lack the awareness of fungal diseases impact, adopted diagnostic portfolio, appropriate early management, and antifungal stewardship. Moreover, ignoring the country based epidemiological studies hinders improving proper management of fungal diseases affecting Egyptian population. The first and only study estimating the burden of serious fungal infections in Egypt was reported in 2017 and 4127 cases of candidemia were estimated using the low international average rate of 5/100,000 (Zaki &Denning, 2017).

Even though epidemiological studies for fungal diseases in Egypt are neglected like many developing countries, this study was conducted in an attempt to give clear picture of the *Candida* bloodstream infection and the circulating *Candida* spp. in the ICU of a tertiary care university hospital in Cairo referring to 30,000 inhabitants per year and estimate the real burden of ICU-acquired candidemia.

#### **Materials and Methods**

Study population

This laboratory-based study included ICU hospitalized patients and conducted between January 2013 to December 2017 at Ain Shams University Specialized Hospital located in Cairo. The study was institutionally approved and implemented under the provisions of the Helsinki Declaration. The patient's consent was exempted because of the laboratory-based observational design of the study.

Candidemia was defined as one positive blood culture for *Candida* species in clinically suspected ICU patient (De Pauw et al., 2008). Thedemographic characteristics, underlying conditions, treatment options and outcomes were analyzed for proven candidaemia cases.

Sampling, culturing, and strain identification

Blood cultures were performed using the BACTEC automated culture system (Becton, Dickinson) and cultures were kept for 7 days. An inoculum from a single colony of each positive culture was inoculated onto Sabouraud's dextrose agar (SDA) plates, incubated at 35°C for 48hr. Identification of Candida isolates processed by routine methods used in the microbiology laboratory. Each isolate was tested for germ tube formation, inoculated on CHROM Agar Candida plates and for substrate assimilation profiles employing Vitek 2 (BioMérieux, Lyon, France) whenever cards are available. The confirmation of morphological identification of the obtained isolates in addition to molecular identification of some isolates using the sequence analysis of the ITS1 - 5.8s - ITS2 region of rDNA and antifungal susceptibility testing were carried out in the Mycology Laboratory at Ain Shams University after the study period.

Susceptibility testing

Antifungal disk diffusion susceptibility testing

of yeasts CLSI M44A2 method (CLSI, 2009) was used for susceptibility testing of isolated Candida strains against fluconazole, voriconazole. caspofungin and micafungin. Mueller-Hinton agar medium supplemented with 2% glucose and 0.5µg of methylene blue per ml was poured in plates at a depth of 4mm. The working solution of individual Candida strains was adjusted to the turbidity of a 0.5MacFarland standard and the agar surface was inoculated by using a swab dipped in the working solution. fluconazole (25µg), voriconazole (1µg), caspofungin (5µg) ready to use discs (@ Liofilchem® s.r.l. Rosetodegli Abruzzi, Italy), andsterile paper disks (Lot no. 355369; HIMEDIA - Mumbai, India) impregnated with 10 µg micafungin (Astellas Toyama Co. Ltd. Japan - Batch no. 3230; HIKMA pharmaceuticals - Amman, Jordan) were placed onto the surfaces of the inoculated plates, and the plates were incubated at 35°C and read after 18 - 24 h by measuring the zone diameters using digital ruler. The interpretive criteria for the tested antifungals were those of CLSI M60 (CLSI, 2017).

Statistical analysis

The incidence of candidemia was expressed in episodes per 1000 ICU admission and 100,000 inhabitants per year. Descriptive analysis was presented as frequencies and confidence interval (CI); medians and range; or means and standard deviation (SD), as appropriate. Univariate analysis was based on contingency coefficient for categorical variables. Pearson's Chi-Square test was used to determine the significant correlation between isolated Candida spp. and underlying conditions. The predictive model for isolated Candida spp. against 4 antifungals was developed using multinomial logistic regression depending on C. albicans as reference. The goodness of fit test for the predictive model is based on Deviance Statistics, anditsfitting criteria are based on likelihood ratio test. Two tailed P-value<0.05 were taken as significant. Data were analyzed using SPSS statistical software (IBM SPSS: version 25).

#### Results

During the study period, a total of 50 proven candidemia episodes were recorded. The incidence rate is 3.3 cases/1,000 ICU admission-year amounting to a burden of 33.33 cases/100,000 inhabitants-year (95% CI 32.97 – 33.70).

All patients were older than 50 years with a median age of 59.50 years (interquartile range: 55 – 62.50; mean±SD 58.93±3.22). 29 (58%) were male while 21 (42%) were female. The highest candidemia episodes 15 (30%) were recorded in neutropenic patients while the lowest incidence was recorded in patients with renal failure 3 (6%). Nine episodes (18%) were proven in cardiovascular patients followed by 7 episodes (14%) in both malignancy and surgery patients; 5 episodes (10%) in respiratory diseases; and 4 episodes were reported in diabetic patients (Table 1).

Of the 50 *Candida* species isolated, *C. albicans* accounted for (n= 13, 26%) while nonalbicans species accounted for (n= 37, 74%). The rank order of *Candida* spp. isolated was *C. krusei* (n= 14, 28%), *C. albicans* (n= 13, 26%), *C. parapsilosis* (n= 10, 20%), *C. tropicalis* (n= 8, 16%) and *C. glabrata* (n= 5, 10%).

More importantly, *C. albicans* showed the highest incidence in malignancy and respiratory diseases patients by 3 cases each, followed by neutropenia and cardiovascular patients by 2 cases each; and one case in each of surgery, diabetes mellitus, and renal failure patients. The highest incidence of *C. krusei* (4 cases) was reported in

neutropenic patients; (3 cases) in malignancy; (2 cases) in each of cardiovascular and respiratory disease; and one case in each of surgery, diabetes mellitus, and renal failure. Three strains of *C. parapsilosis* were isolated from neutropenic patients; 2 from cardiovascular and surgery patients; while only onewas isolated from each of malignancy, diabetes mellitus and renal failure. *C. tropicalis* was isolated from neutropenic patients (3 cases); cardiovascular and surgery (2 cases each) and one case in diabetes mellitus. *C. glabrata* isolated mainly from neutropenic patients (3 cases) and one case only from each of cardiovascular and surgery patients.

Concerning Candida species richness, the data obtained revealed that the five isolated Candida species were identified in neutropenia, cardiovascular and surgery patients' episodes, while only C. albicans and C. krusei, were isolated from respiratory diseases patients. Four species were identified in diabetes mellitus and three species were identified in malignancy and renal failure patients' episodes. The correlation between isolated Candida spp. and underlying conditions were (44.7%; P-value: 0.928) which indicates that there is no significance between isolated Candida spp. and underlying conditions.

TABLE 1. Characteristics of the proven candidemia episodes.

Underlying condition	N (%)	Ger	ıder						
		Male	Female	Mean age±SD	C. albicans	C. krusei	C. parapsilosis	C. tropicalis	C. glabrata
Neutropenia	15 (30%)	9 (60%)	6 (40%)	59.5±12.02	2 (13.3)	4 (26.6)	3 (20)	3 (20)	3 (20)
Cardiovascular	9 (18%)	5 (55.5%)	4 (44.5%)	62.5±13.44	2 (22.2)	2 (22.2)	2 (22.2)	2 (22.2)	1 (11.1)
Malignancy	7 (14%)	3 (42.9%)	4 (57.1%)	55±7.07	3 (42.8)	3 (42.8)	1 (14.2)	0	0
Surgery	7 (14%)	5 (71.4%)	2 (28.6%)	54.5±6.36	1 (14.2)	1 (14.2)	2 (28.5)	2 (28.5)	1 (14.2)
Respiratory diseases	5 (10%)	1 (20%)	4 (80%)	62.50±10.61	3 (60)	2 (40)	0	0	0
Diabetes mellites	4 (8%)	3 (75%)	1 (25%)	60±4.24	1 (25)	1 (25)	1 (25)	1 (25)	0
Renal failure	3 (6%)	3 (100%)	0 (0%)	58.50±3.54	1 (33.3)	1 (33.3)	1 (33.3)	0	0
Total	50 (100%)	29 (58%)	21 (42%)	58.93±3.22	13 (26%)	14 (28%)	10 (20%)	8 (16%)	5 (10%)

Empirical treatment with fluconazole and/ or voriconazole is the only choice used when a positive episode of candidemia is reported in our hospital due to the unavailability of other antifungals. The overall recorded mortality of candidemia patients during the study period were 13 cases (26%).

The antifungal susceptibility profiles of the isolated Candida spp. are presented in Table 2. All C. albicans, C. tropicalis, C. parapsilosis strains were susceptible to the 4 antifungal drugs tested. The 14 strains of C. krusei were resistant to fluconazole. The 5 strains of C. glabrata were resistant to voriconazole and 3 strains (60%) were susceptible dose dependent (SDD) to fluconazole. C. albicans strains showed highest sensitivity against caspofungin (76.9%) followed by micafungin (69.3%) whereas lowest sensitivity was recorded against both voriconazole and fluconazole (61.5%). C. krusei was highly sensitive to caspofungin (85.7%), followed by voriconazole (78.5%) and finally, micafungin (64.3%) and all strains were resistant to fluconazole. As for, C. parapsilosis, it showed high sensitivity against voriconazole (90%), then fluconazole (80%) and (70%) for both caspofungin and micafungin. C. tropicalis was most susceptible to voriconazole (87.5%) and least susceptible to caspofungin (62.5%) and had similar activity (75%) against both micafungin and fluconazole. Finally, C. glabrata showed highest sensitivity against both caspofungin and micafungin (80%) followed by fluconazole (60%) and all strains were resistant to voriconazole. It is worth mentioning that statistically caspofungin and micafungin were not significant for species (P-value>0.05) while voriconazole and fluconazole were significant for species (P-value<0.05).

#### **Discussion**

Egypt is still late on proper management of fungal diseases among its hundred million population as health settings ignore investigations of fungal infection among risk groups of patients. *Candida* infection has always been identified unintentionally and the antifungal susceptibility profile for the isolated *Candida* spp. is not carried out. This study is a single center study analyzing only the first recorded episode of bloodstream infection indicated by positive blood culture after ICU admission. Therefore, we emphasize that candidemia incidence is underestimated. To the

best of our knowledge, this is the first report on ICU acquired candidemia in Egypt.

In this study, candidemia incidence rate was 3.3 episodes per 1,000 ICU admissions with an estimated country burden of 33.3 per 100,000 inhabitants. The highest incidence rate per 1,000 ICU admissions previously reported was 34.3 episodes and the highest country burden of candidemia was 21 episodes per 100,000 inhabitants (González de Molina et al., 2012 and Bongomin et al., 2017). According to the results obtained, Egypt showed the highest candidemia burden compared to the other Middle East neighboring countries. Candidemia burden in Israel is 11/100,000 inhabitants while in Qatar is 15.4/100,000 inhabitants (Ben-Ami & Denning, 2015 and Taj-Aldeen et al., 2015).

The most recent epidemiological reports in Middle East countries indicated that Nonalbicans Candida species are highly incident than Candida albicans, as the lowest incidence reported was in Turkey by 45.4% and the highest incidence was reported from Iran by 72.7%, while the incidence was 55%, 55.6%, 65.9%, 66.2%, and 68% in United Arab Emirates, Israel, Saudi Arabia, Qatar, and Kuwait, respectively. Our results revealed that the incidence of Nonalbicans Candida species is the highest by 74% if compared with other countries in the Middle East. Non-albicans Candida spp. (C. parapsilosis, C. tropicalis and C. glabrata) were recorded in this study as well as in other Middle East countries while C. krusei recorded in Israel, Saudi Arabia and in this report only. It is noteworthy to mention that C. parapsilosis dominated as a leading agent of candidemia episodes in 3 countries (Iran, Kuwait, and Israel), C. glabrata dominated in 2 countries (Qatar and Turkey) and C. krusei dominated in our study only (Yeşilkaya et al., 2017; Vaezi et al., 2017; Ellis et al., 2003; Ben-Ami et al., 2013; Al Thaqafi et al., 2014; Taj-Aldeen et al., 2014 and Alobaid, 2017).

The predominance of *C. krusei* in our study (28%) logically attributed to the excessive using of fluconazole as the only available antifungal drug of candidemia treatment in our hospital and absence of physician's awareness of the inherent resistance of *C. krusei* to azoles (Antinori et al., 2016). *C. parapsilosis* comes next (20%) reflecting the weakness of the infection control measures while *C. tropicalis* higher in proportion than

*C. glabrata* which is more consistent with the explanation given previously that *C. tropicalis* is more common in warm climates countries (Vaezi et al., 2017 and Tan et al., 2015).

The antifungal susceptibility testing in this study revealed that all isolated *Candida* spp. exhibited variable resistance rates to the 4 tested antifungal agents, as low rates of resistant isolates were observed for caspofungin (24%) while the high rates were observed for fluconazole (50%). Overall, echinocandins exhibited higher activity than azoles against all isolates suggesting the

possibility for alternative treatments in our hospital. Inappropriate use of antifungal agents causes antifungal resistance and the uninfected patients faces unneeded toxicity and expenses. Therefore, antifungal stewardship teams must understand the local epidemiology, frequency of occurrence and antifungal susceptibility profile in order to guide the clinicians in diagnosing and treating nosocomial candidiasis (Pfaller & Castanheira, 2016; Alexander et al., 2013; Ruhnke, 2014; Shields et al., 2013 and Kullberg & Arendrup, 2015).

TABLE 2. In vitro susceptibility of isolated Candida spp. against 4 antifungals (CLSI M60).

Antifungal	Species	S (%)	I (%)	SDD (%)	Total (%)	R (%)	OR	95 % CI [Lower, Upper]	P-value
	C. albicans	9 (69.2)	1(7.7)	0	10 (76.9)	3 (23.1)	1		
Caspofungin (CFG)	C. krusei	10 (71.4)	2 (14.3)	0	12 (85.7)	2 (14.3)	0.871	[0.452, 1.680]	
	C. parapsilosis	7 (70)	0	0	7 (70)	3 (30)	1.081	[0.560, 2.088]	0.887
	C. tropicalis	5 (62.5)	0	0	5 (62.5)	3 (37.5)	1.218	[0.618, 2.400]	
	C. glabrata	4(80)	0	0	4 (80)	1 (20)	0.890	[0.359, 2.208]	
Micafungin (MFG)	C. albicans	6 (46.2)	3 (23.1)	0	9 (69.3)	4 (30.7)	1		
	C. krusei	7 (50)	2 (14.2)	0	9 (64.3)	5 (35.7)	1.034	[0.791, 1.350]	
	C. parapsilosis	6 (60)	1 (10)	0	7 (70)	3(30)	0.916	[0.678, 1.236]	0.958
	C. tropicalis	5 (62.5)	1 (12.5)	0	6 (75)	2 (25)	0.847	[0.609, 1.179]	
	C. glabrata	3 (60)	1 (20)	0	4 (80)	1(20)	0.806	[0.540, 1.202]	
Voriconazole (VCZ)	C. albicans	7 (43.7)	1 (7.7)	0	8 (61.6)	5 (38.4)	1		
	C. krusei	11 (78.5)	0	0	11 (78.5)	3 (21.5)	0.713	[0.510, 0.998]	
	C. parapsilosis	8 (80)	1 (10)	0	9 (90)	1 (10)	0.577	[0.375, 0.887]	< 0.001*
	C. tropicalis	6 (75)	1(11.1)	0	7 (87.5)	1 (12.5)	0.636	[0.413, 0.981]	
	C. glabrata	0	0	0	0	5 (100)	0	0	
Fluconazole (FCZ)	C. albicans	6 (46.1)	0	2 (15.4)	8 (61.5)	5 (38.5)	1		
	C. krusei	0	0	0	0	14(100)	0	0	
	C. parapsilosis	7 (70)	0	1 (10)	8 (80)	2 (20)	0.787	[0.414, 1.498]	< 0.001*
	C. tropicalis	5 (62.5)	0	1 (12.5)	6 (75)	2 (25)	0.884	[0.454, 1.723]	
	C. glabrata	0	0	3 (60)	3 (60)	2 (40)	2.044	[0.770, 5.423]	

#### Conclusion

This study sheds light on the candidemia incidence rate and the circulating Candida spp. in a university tertiary care hospital in Egypt for the first time. The results obtained indicated that the incidence rate of candidemia is the highest compared with neighboring countries in the Middle East. Putting in mind the limitations of this study; it was in one medical center with low number of cases, the inability to properly assess the risk factors and management actions as well as the patient's outcome since this study was laboratory based. Nevertheless, the results obtained are giving an alarm to the Egyptian authorities about the fact of the complete ignorance of proper management of fungal diseases in health settings. The policymakers and health settings in Egypt should speed up the procedures to ensure proper management of fungal diseases by; firstly, adopting a well-developed diagnostic portfolio. Secondly, raising awareness and the continuous training of physicians and nursing staff on methods of fungal diseases management. Thirdly, the merging control of fungal diseases in infection control programs. Fourthly, activating the antifungal stewardship programs in all health settings. Finally, promoting and funding of epidemiological studies of fungal diseases at the level of the whole country.

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# معدل حدوث مرض الكانديدميا المكتسب في الرعاية المركزة في مستشفي رعاية ثلاثية في القاهرة \_ مصر

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تهدف هذه الدراسة المعملية إلى تحديد معدل الإصابة بمرض كانديدا الدم المكتسب في وحدة العناية المركزة وتحديد أنواع الكانديدا المسببة للمرض في مستشفى الرعاية الثلاثية الجامعي لأول مرة في مصر. أجريت هذه الدراسة بين يناير 2013 إلى ديسمبر 2017 في مستشفى جامعة عين شمس التخصصي في القاهرة. معدل الإصابة وتوزيع الأنواع وكذلك تم تحديد اختبار الحساسية المضادة للفطريات من أنواع الكانديدا المعزولة ضد الفلوكونازول، الكاسبوفونغين، والميكافونجين. خلال فترة الدراسة، تم تسجيل ما مجموعه 50 حلقة أثبتت الإصابة بمرض كانديدا الدم. معدل الإصابة هو 3.3 حالات/1000 في السنة في مرضى وحدة العناية المركزة تبلغ عبئها 33.33 حالة/00000 نسمة في السنة (%95 م 33.70 و 33.70). من بين 50 نوعًا من أنواع الكانديدا المعزولة ، كانت C. Albicans تمثل %26 بينما كانت الأنواع غير الألبيكنية %74. ترتيب رتبة الكانديدا المعزولة ، كان:

(C. krusei 14 (28%), C. albicans 13 (26%), C. parapsilosis 10 (20%), C. tropicalis 8 (16%) and C. glabrata 5 (10%)).

كشفت الإختبارات أن جميع أنواع الكانديدا المعزولة قد أظهرت معدلات مقاومة متغيرة للعوامل الأربعة المضادة للفطريات حيث لوحظت معدلات منخفضة من العزلات المقاومة للكاسبوفونغين (%24) في حين لوحظت معدلات عالية للفلوكونازول (%50). أشارت النتائج التي تم الحصول عليها إلى أن معدل حدوث الإصابة بمرض كانديدا الدم المكتسب ومعدل ظهور الأنواع الغير الألبيكنية هو الأعلى مقارنة بالدول المجاورة في الشرق الأوسط.