

ORIGINAL ARTICLE

Candida Glabrata fungemia: An emerging threat in Egypt

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ABSTRACT**Key words:***Candida glabrata*,
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Background: During the past decade, *Candida glabrata* (*C. glabrata*) has emerged as an important cause of fungemia. **Objectives:** The aim of this study is to isolate strains of *C. glabrata*, determine its incidence and antifungal susceptibility in ICU patients attending Benha University Hospital. **Methodology:** This study was carried out in the period from January 2016 to December 2018. Blood culture was done to patients with suspected blood stream infections. Isolation of candida and identification of *C. glabrata* by phenotypic and genotypic methods then antifungal susceptibility testing was done. **Results:** Fourty three strains of candida were detected of which 9 strains were *C. glabrata* (21%). our study shows that the most common underlying risk factors in our institution were neutropenia (7cases), malignancy (6 cases), respiratory diseases (3cases), treatment with central venous catheter (3cases), receipt of parenteral nutrition (2cases) and exposure to surgery (2cases). *C. glabrata* was highly sensitive to caspofungin 7 (77.8%) followed by amphotricin B 6 (66.7%), fluconazol 2 (22.2%) with the least sensitivity to voriconazol 1 (11.1%). **Conclusion:** *C. glabrata* is a common cause of candidemia in our ICU. Neutropenia, malignancy, respiratory disease and treatment with central venous catheter are risk factors for its acquisition.

INTRODUCTION

Bloodstream infections (BSI) are a major cause of morbidity and mortality worldwide particularly among the intensive care units (ICU) patients¹. Candidaemia incidence has been increased in the past few decades with geographical variation in its epidemiology as a result of excessive use of broad-spectrum antibiotics, immuno-suppressive drugs and indwelling devices². Different candida species represent the 4th most common cause of nosocomial BSI and the 3rd common cause in ICU- BSI with the highest mortality rate among all causes of BSI³. Although *Candida albicans* remains a major cause of candidiasis, non albicans species especially *C. glabrata* have emerged as an important pathogens⁴. *C. glabrata* becomes the second most common cause of invasive candida infections preceded only by *C.albicans*⁵. Multidrug resistant *C. glabrata* strains have been reported in the last few years that may participate to some extent in increasing the incidence of *C. glabrata* infections⁶.

The aim of this study was to isolate strains of *Candida glabrata*, determine its incidence and antifungal susceptibility in ICU patients attending Benha University Hospital.

METHODOLOGY

This study was approved by the Ethical Committee of Benha Faculty of Medicine and a written informed consent was obtained from all patients participate in this study.

The study was carried out in the period from January 2016 to December 2018, conducted to 450 patients admitted at Benha University Hospital adult ICU and suspected by the resident physician to have blood stream infection.

Sample collection:

Following universal precautions, 10 ml venous blood was collected, inoculated immediately into BacT/ALERT blood culture bottles containing 32 ml of complex media and 8 ml of a charcoal suspension, an automated blood culture system (BacT/ALERT, Biomerieux) was used for blood culture.

Phenotypic identification by the traditional methods:

Subcultures: using sterile syringes were done on Sabouraud dextrose agar (SDA) (supplemented with 50 µg/ml of chloramphenicol and 5 µg/ml of gentamicin), incubated at 37 °C for 24 - 48 h. Tiny, white to creamy, glossy, smooth and convex colonies that remain small despite further incubation were shown .

Microscopic examination: using Gram staining showed small spherical rather than oval yeast cells, with single budding that do not form hyphae or pseudo-hyphae.

Germ tube test: was carried out by suspending a colony from the growth into 0.5 ml of human serum in a test tube, incubated for 2-3 hours at 35-37°C, it was negative.

Sugar fermentation tests: for glucose, lactose, maltose, mannite, sucrose and trehalose were done by adding 1% of each sugar in a separate test tube (with inverted Derhum's tube) containing peptone water and 1% Andrad's indicator, incubated for 24 hours at 37 °C, glucose and trehalose were fermented with production

of acid and gas. The isolated strains were stored at -80°C for further identification, confirmation and antifungal susceptibility tests.

Molecular identification by Polymerase chain reaction (PCR):

DNA extraction and purification:

DNA was extracted using G-Spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Korea) following the manufacture instructions.

Nucleic acid amplification: was carried out in a total volume of 50µL containing approximately 5µl of the purified DNA template, 25µl of 2×EasyTaq® PCR SuperMix, 1µl of the forward primer (5-CCCAAAAATGGCCGTAAGTATG-3), 1µl of the reverse primer

(5-ATAGTCGCTACTAATATCACACC-3), the volume was completed by nuclease free water and the mixture was kept in a PCR tube.

The PCR cycle parameters were as follows; initial denaturation (one cycle at 94°C for 5 min.), denaturation (35 cycles at 94°C for 30 sec.), annealing (35 cycles at 51°C for 30 sec.), extension (35 cycles at 72°C for 1 min), followed by final extension (one cycle at 72°C for 10 min.).

Detection of PCR products: 2% agarose gel stained with ethidium bromide solution (concentration of 0.5 µg/ml) in 1X TBE buffer at 80 V for 60 min was used to analyze the PCR products. 100bp ladder DNA marker was run with amplified products for sizing of the bands. Gels were then visualized with a UV transilluminator (figure 1).



Fig. 1: lanes 2, 4 and 7 show bands at 674bp suggestive of *C. glabrata*

Antifungal susceptibility testing: was performed by disc diffusion method for fluconazole (25µg), amphotericin B (50µg), voriconazole (1µg) [obtained from Hi-Media, Mumbai] and caspofungin (5µg) [obtained from Kairosafe, Italy] on Mueller Hinton Agar containing 2% glucose and 0.5µg/ml methylene blue according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for antifungal disc diffusion susceptibility testing of yeasts (CLSI document-M44). Results were expressed as sensitive, intermediate resistant or resistant to the tested antifungal drug according to the Zone Diameter Interpretive Standards-CLSI^{7,8}.

Statistical analysis:

Data were analyzed using SPSS statistical software (IBM SPSS: version 21). Fisher exact test was applied

for comparison of categorical variables. $P < 0.05$ was considered as statistically significant. Descriptive analysis was presented as frequencies, confidence interval (CI), Odd's ratio (OR), mean and standard deviation (SD).

RESULTS

The present study includes all fungemia cases that occurred from January 2016 till December 2018. There were 43 fungemia cases of which *C. glabrata* fungemias accounted for 9 cases (21 %).

C. glabrata fungemias occurred in 5 (55.5%) men and 4 (44.4%) women, their mean age was (62.77 ± 10.69) of which 6 subjects (66.6%) were > 60 years and 3 subjects (33.3%) were between 41 and 60 years.

Table 1: Demographic and clinical characteristics of patients with *C. glabrata* fungemia

Variables	<i>Candida glabrata</i> (N=9)	Other candida species (N=34)	Total (N=43)	Test of significance "FET"	OR & 95% CI	P value
Sex						
Male	5 (20.0%)	20 (80.0%)	25 (100%)	.031	.875 (.199-3.850)	1.000
Female	4 (22.2%)	14 (77.8%)	18 (100%)			
Respiratory diseases	3 (23.1%) (7% of total candida)	10 (76.9%)	13 (100%)	.052	1.2 (.250-5.768)	1.000
Renal diseases	0 (0.0)	5 (100%)	5 (100%)	1.498	-	.566
Malignancies	6 (40.0%) (14% of total candida)	9 (60.0%)	15 (100%)	5.062	5.5 (1.14-27.01)	.046*
D.M.	3 (12.5%) (7% of total candida)	21 (87.5%)	24 (100%)	2.33	.310 (.066-1.457)	.153
Cardiovascular diseases	1(16.7%) (2.3% of total candida)	5 (83.3%)	6 (100%)	.077	.72 (.074-7.125)	1.000
Cirrhosis	0 (0.0)	7 (100%)	7 (100%)	2.21	-	.314
Surgery	2 (28.6%) (4.7% of total candida)	5 (71.4%)	7 (100%)	.295	1.657 (.264-10.39)	.624
Systemic antibiotics	9 (22.5%) (20% of total candida)	31(77.5%)	40 (100%)	.854	.775 (.656-916)	1.000
Treatment with central venous catheter	3 (25.0%) (7% of total candida)	9 (75.0%)	12 (100%)	.167	1.389 (.286-6.753)	.692
Neutropenia	7 (41.2%) (16.3% of total candida)	10 (58.8%)	17 (100%)	6.964	8.4 (1.48-47.66)	.018*
Receipt of parenteral nutrition	2 (25.0%) (4.7% of total candida)	6 (75.0%)	8 (100%)	.098	1.333 (.220-8.082)	1.000

The majority of patients had multiple underlying illness and risk factors that have been associated with fungemia (table1). The most common underlying risk factors in our institution were neutropenia (7 cases), malignancy (6 cases), respiratory diseases (3 cases), treatment with central venous catheter (3 cases), receipt of parenteral nutrition (2 cases) and exposure to surgery (2 cases).

Empirical treatment with fluconazole is the only choice used when a positive case of candidemia is reported in the hospital due to unavailability of other costly antifungals.

The antifungal susceptibility testing of the isolated *Candida glabrata* is presented in (table 2). *C. glabrata* was highly sensitive to caspofungin 7 (77.8%) followed by amphotericin B 6 (66.7%), fluconazole 2 (22.2%) with the least sensitivity to voriconazole 1 (11.1%).

Table 2: The antifungal susceptibility testing of the isolated *C. glabrata*

Drug		Fluconazol	Voriconazole	Amphotricin B	Caspofungin
Sensitivity	Sensitive	2 (22.2%)	1 (11.1%)	6 (66.7%)	7 (77.8%)
	Intermediately resistant	1 (11.1%)	0 (0.0%)	1 (11.1%)	1 (11.1%)
	Resistant	6 (66.7%)	8 (88.9%)	2 (22.2%)	1 (11.1%)
<i>C. glabrata</i> (N=9)		9	9	9	9

DISCUSSION

C. glabrata is reported with increasing frequency as a cause of fungemia and invasive candidiasis. In our study, *C. glabrata* represents about 21% of all candidemias. In Egypt, a study was conducted between 2013 and 2017 at Ain Shams University specialized Hospital, *C. glabrata* incidence was 10% of all isolated *Candida*⁹. Another study was carried out in pediatric ICU of Mansoura University Children's Hospital, *C.*

glabrata incidence was 8%¹⁰. The rate in this study is consistent with rates reported in other studies in middle east countries (Turkey (18%)¹¹- Iran (22.7%)¹²). While in United Arab Emirates, Kuwait and Saudi Arabia the rate is less (5%)¹³, (5.6%)¹⁴, (16.3%)¹⁵ respectively. A higher incidence is reported in Qatar (25.5%)¹⁶. Worldwide, different rates were recorded (Taiwan (30%)¹⁷- Brazil (3-13%)¹⁸- Peru (9.5%)¹⁹- Korea (4.6%)²⁰- Michigan (17.5%)²¹- India (18%)²²).

In our study, *C. glabrata* candidemia was seen more in male patients than females. Some studies had reported an almost equal incidence among males and females^{17,20}.

In the present study, *C. glabrata* fungemia is more common in elderly people. The mean age is 62.77±10.69 years. Gupta et al.²¹ and Ruan et al.¹⁷ also report this finding. This association may be due to underlying disease or antifungal drug resistance. Lockhart et al.²³ found increased mucosal colonization with *C. glabrata* among healthy elderly individuals. Several reports have focused attention on the increasing rate of colonization and infection with the natively resistant candida species (*C. krusei* and *C. glabrata*) in patients receiving fluconazole^{24,25}.

As regard the underlying illness and probable risk factors, our study showed that the most common underlying risk factors in our institution were neutropenia (7 cases), malignancy (6 cases), respiratory diseases (3 cases), treatment with central venous catheter (3 cases), receipt of parenteral nutrition (2 cases) and exposure to surgery (2 cases). All patients with *C. glabrata* candidemia were treated with broad spectrum antibiotics.

Abass et al.⁹ reported that 20% of *C. glabrata* were isolated from neutropenic patients. Gupta et al.²¹ showed that all the patients in their study were on broad spectrum antimicrobials and had central venous catheter, (26.9%) patients were on hemodialysis due to renal failure and (11.5%) patients received total parenteral nutrition¹⁶. Ruan et al. reports that the most common underlying diseases in these patients were cancer (49%), D.M. (34%) and renal failure (25%). The most common risk factors were the use of central venous catheter (88%), antimicrobial treatment (87%) and parenteral nutrition (51%). Malani et al.¹⁹ revealed that the most common predisposing factors were use of broad spectrum antibiotics (86% of cases), use of central venous catheters (77%), renal failure (46%) and receipt of parenteral nutrition (45%) . Choi et al.²⁰ reported renal insufficiency as a an important risk factor¹⁸.

As regard the antifungal susceptibility testing of the isolated *C. glabrata*, the strains showed the highest sensitivity to caspofungin 7 (77.8%) and the least sensitivity to fluconazol 2 (22.2%) and voriconazol 1 (11.1%). Abass et al.⁹ reported that all (100%) *C. glabrata* strains were resistant to voriconazol while 40% were resistant to fluconazol and 80% were sensitive to caspofungin. Malani et al.¹⁹ showed that 60% of isolates were resistant to fluconazol and 44% were resistant to voriconazole. In a study by Tumbarello et al.²⁶, *C. glabrata* isolates were assumed to be inherently more resistant than *C. albicans* to fluconazole. Ruan et al.¹⁷ reported that rates of antifungal susceptibility were 63% for fluconazole, 93% for voriconazole, 96% for caspofungin and 98% for amphotericin B. On the other

side, Mokaddas et al.¹⁴, showed low resistance to fluconazol (5.8%) and all strains were susceptible to amphotericin B and voriconazol. Ormani et al.¹⁵ reported that the majority of the tested isolates were susceptible to voriconazol and amphotericin B while 91% were susceptible to fluconazol.

There are some limitations in our study. First, the sample size was limited as the data is from a single place (ICU) in a single institution and data could be influenced by local infection control measures and local antibiotic policies. Second, small sample size precludes meaningful statistical analysis of risk factors that increase acquisition of *C. glabrata* fungemia. Finally, the incidence of candidemia is underestimated in Egypt as health care settings don't perform routine investigations of fungal infections among high risk group patients.

CONCLUSION

C. glabrata is a common cause of candidemia in our ICU. Neutropenia, malignancy, respiratory disease and treatment with central venous catheter are risk factors for its acquisition. Great resistance to azoles should be put in consideration when treat such infections. We recommend further studies to include other hospitals in our governorate and identify other common non albicans species, increase awareness between physicians about methods of fungal management and merging control of fungal diseases in infection control programs.

Conflicts of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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