Microbes and Infectious Diseases 2025; 6(1): 389-395

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Prevalence of *Candida* species colonization in patients with chronic liver disease admitted to the intensive care unit: A prospective two center study

Eman Medhat Hasan[1], Magdy Amin El Serafy[1], Rasha Eletreby[1], Hend Ibrahim Shousha[1], Mona Abdelaziz Wassef[2], Sherifa Tarek Salem[2], Rana Mostafa Anwar*[3], Nermin Zaky Mostafa[4] , Basma Ahmed Elawady[4]

1) *Department of Endemic Medicine and Hepatogastroenterology, Faculty of Medicine, Cairo University.*
2) *Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University.*
3) *Department of Hepatogastroenterology and Endoscopy, Agouza Police hospital.*
4) *Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University.*

ARTICLE INFO

Article history:
Received 25 October 2024
Received in revised form 29 November 2024
Accepted 8 December 2024

Keywords:
Candida
Colonization
Hepatic dysfunction
Antifungal resistance

ABSTRACT

Background: Due to their weakened immune systems, patients with chronic liver illness are more vulnerable to a variety of opportunistic fungal infections. *Candida* species are commonly isolated from patients admitted to hepatic intensive care unit (ICU). **Aim:** to detect the prevalence of *Candida* species colonization and their antifungal susceptibility in ICU patients with chronic liver disease. **Methods:** Two-center case control study was carried out from January 2019 to June 2020 on 100 cirrhotic patients with chronic hepatitis C virus (HCV) admitted to hepatic ICU. Oral swabs and urine samples were cultured on CHROM agar followed by an automated VITEK 2 technique for identification of *Candida* species and antifungal susceptibility testing. **Results:** *Candida albicans* was the most prevalent *Candida* species in both urine (74%) and oral swab (66%) cultures using CHROM agar. The most prevalent *candida* species detected with an automated VITEK 2 technique was *Candida albicans* in both urine and oral swab cultures with higher resistance to fluconazole in isolates from urine and higher resistance to caspofungin in mouth swabs. **Conclusion:** *Candida albicans* is highly prevalent in cirrhotic patients admitted to the ICU with 3-4 times increased risk of colonization compared to controls with multiple antifungal drug resistance. Voriconazole has high efficacy against *Candida* species with low resistance and can be considered in critically ill patients to offer better survival. Strict follow up for critically ill cirrhotic patients admitted to ICUs for early diagnosis and treatment of fungal infections to offer better survival.

Introduction

In critically sick patients, multiple-site colonization with *Candida* species is well acknowledged as a significant risk factor for invasive fungal infections, and the colonization density may serve as a predictor of systemic candidiasis. *Candida* infections, especially in immunocompromised patients, are a leading cause of morbidity and mortality because of the limited availability of antifungal medications and rise in antibiotic resistance. Early implementation of suitable diagnostic and treatment interventions directly affects the course and outcome of the patient's condition [1-3]. Due to the inappropriate

DOI: 10.21608/MID.2024.331260.2311
* *Corresponding author:*Rana Mostafa Anwar
E-mail address: *ranaty2rony@yahoo.com*

use of antibiotics and the implantation of invasive procedures, particularly in patients in the intensive care unit (ICU), the prevalence of candidiasis has significantly increased. The majority of superficial and systemic infections were caused by *Candida albicans*; however, the frequency of infections caused by *non-albicans* species, such as *Candida glabrata, Candida krusei, Candida tropicalis, Candida parapsilosis, Candida guilliermondii, and Candida dubliniensis,* has considerably grown [4].

Accurate and early detection of colonization are essential prerequisites for the right kind of treatment response [4]. The aim of this study was to detect the prevalence of *Candida* colonization and antifungal susceptibility in ICU patients with chronic liver disease.

Methods

A cross-sectional, prospective study was conducted in Endemic Medicine Department, Cairo University and the Gastroenterology and Hepatology Department at El Agouza Police Hospital from January 2019 to June 2020. The study included 100 adult patients (18-70 years old patients) with HCV related chronic liver disease admitted in the hepatic intensive care units in addition to 20 healthy individuals as control. Informed consent was obtained from all patients or their relatives (if severely ill to consent) before the start of any study related procedure.

Full history, clinical examination and routine laboratory work up for decompensated liver disease were done. In addition to urine cultures and mouth swabs were performed.

Both urine and mouth samples were collected under strict aseptic conditions. Mouth swabs and urine samples were cultured on BBL™ (Baltimore Biological Laboratory) CHROM agar™ *Candida* media (Becton Dickinson, Germany) [5-7].

Every plate was incubated inverted for 20 to 48 hours at 35º ± 2ºC in an aerobic environment. Fresh subculture of *Candida* isolates obtained from patients was done on Sabouraud dextrose agar and incubated aerobically at 35º ± 2ºC for 24 hours. A suitable number of colonies of a pure culture were transferred and suspended in 3.0 mL of sterile saline using a sterile swab, and the turbidity was adjusted to meet the McFarland 2.0 standard. Reagent cards "VITEK® 2 YST ID cards" (BioMérieux, Marcy-l'Etoile, France) samples were examined as illustrated in the manufacture instructions.

Based on the instructions provided by an automated VITEK 2 compact system, yeast antifungal susceptibility testing (AST) card called "AST-YS08" (BioMérieux, Marcy-l'Etoile, France) was carried out [8]. The Clinical and Laboratory Standards Institute's (CLSI) clinical breakpoints were used to evaluate all MIC values [9].

Demographic and laboratory data of patients versus control, fungal cultures result in both mouth and urine swabs, and antifungal sensitivities of *Candida* species were studied and compared

Sample size and technique:

The Power Analysis and Sample Size (PASS) Software was used to calculate the sample size of this cross-sectional study with confidence interval 4. The sample size was 80 participants with a confidence interval of 95% and 5% margin of error. Considering dropout rates of 10%, the final sample size was 100 participants.

Statistical methods:

Through the use of a Microsoft Excel workbook file, the data was uploaded into a personal computer. The SPSS software was used to verify the data for flaws and completeness (statistical package for social science version 23). Version 23 of the SPSS application was used for data analysis. P values less than 0.05 are the threshold for statistical significance in this investigation. The student "t" test, one-way analysis of variance, and Chi-squared test were the statistical tests that were employed.

Results

Among the studied group, 76% of the patients were males. The mean age of the studied patients was 56.3 years. Patients had lower mean hemoglobin, total leucocytic count, platelet count, albumin, mean CRP, ESR, liver enzymes, total bilirubin, urea, creatinine and INR compared to controls and results were highly statistically significant with P<0.01 by chi square test (**Table 1**).

Colonies of *Candida albicans* on CHROM agar appeared light to medium green after incubation while colonies of *Candida tropicalis* were blue greenish to metallic-blue and colonies of *Candida krusei* were light rose with a white border. Among the studied group, a higher percentage of positive urinary *Candida albicans* (74%) among cases with liver cirrhosis were detected compared to controls as detected by both CHROM agar and VITEK techniques. The frequency of *C. tropicalis*, *C. parapsilosis*, *C. Krusei* and other uncommon species isolated from urine were 16%, 3%, 17% and

11% respectively. None of the controls had positive urinary culture for these species. Cases with cirrhosis have three times the risk of having *Candida albicans* compared to controls in mouth swab cultures followed by *C. krusei*, C. *tropicalis*, *C. parapsilosis* and other uncommon species in a percentage of 66%, 15% , 14%, 5% and 9% respectively as calculated by odds ratio. Results of *Candida* species isolated from urine samples and mouth swabs are illustrated in **Figure 1.**

Among the 100 urine samples and oral swabs collected, 18 yielded positive results for *Candida* species. Among the 100 urine samples, 21% yielded mixed infections while among the oral swabs 17% mixed infections were detected. In patients, the most prevalent *Candida* species detected with VITEK technique was *Candida albicans* in both urine and mouth swab cultures **(Table 2)** with higher resistance to fluconazole in urine cultures compared to other *Candida* species and the difference was not statistically significant. *Candida albicans* and other *Candida* species isolated from urine cultures showed no resistance for voriconazole, amphotercin B and micafungin but *Candida albicans* showed marked resistance to fluconazole in a percentage of 50% (Chisquare 1.9) and the non albicans showed a significant resistance to caspofungin, fluconazole and fluocytosine in a percentage of 57.1%, 14.3% (chi square 0.9) and 14.3% respectively. *C. albicans* isolated from mouth swab had lower resistance to fluconazole, caspofungin or flucytosine while the non albicans *Candida* showed higher resistance to caspofungin, fluconazole and fluocytosine in a percentage of 33.3%, 16.7% and 16.7% (chi square 1.2) respectively with significant P value <0.05 by chi square test.

However, there was no statistical significance between *Candida albicans* and other *Candida* species isolated from both urine and mouth swab regarding laboratory parameters. Slightly higher AST and ALT values were noted among cases with other *Candida* species compared to *Candida albicans* regarding oral swabs, while higher bilirubin and ESR levels were noted in *non albicans Candida* in urine samples compared to *Candida albicans* with a P value of >0.05 that is not statistically significant **(Figure 2).**

Among patients with positive mouth swab cultures, the highest mortality was for those with *C. parapsilosis* infection (100%), followed by *C. Krusei* (80%) then *C. albicans* (78.8%) and *C. tropicalis* (78.6%). In patients with positive urine cultures, the highest mortality was also for *parapsilosis* (100%) followed by *C. tropicalis* (87.5%), *C. albicans* (78.4%) and C. *Krusei* (70.6%) (**Table 3).** Urine samples and oral swabs with mixed *Candida* species are illustrated in **(Table 4).**

Table 1. Demographic and laboratory data of patients *versus* control

		Patients No.= 100		**Control No.= 20**		
Age /years (mean/SD)		56.3/ 7.3		40.2/ 13.4		
Gender	Male (N. %)	76 (76%)		10 (50%)		
	Female (N. %)	24 (24%)		10 (50%)		
Laboratory parameters		**Patients N.=100**		**Controls N.=20**		***P* value**
		Mean	**SD**	**Mean**	**SD**	
Hemoglobin (g/dl)		9.9	0.5	13.9	0.9	0.000**
Total leucocytic count (X 10^9/L)		3.6	0.4	6.1	1.0	0.000**
Platelets (X 10^3 / mL)		89.9	15.4	346.5	52.2	0.000**
CRP (mg/L)		44.2	19.6	3.6	1.1	0.000**
ESR first hour (mm/ hour)		21.7	5.7	6.1	1.9	0.000**
ESR second hour (mm/hour)		39.2	11.7	14.2	2.4	0.000**
ALT (IU/L)		85.5	19.6	27.6	8.9	0.000**
AST (IU/L)		90.1	14.9	27.1	10.9	0.000**
Total bilirubin (mg/dl)		4.6	1.5	0.6	0.2	0.000**
Albumin (g/dl)		2.4	0.3	3.9	0.2	0.000**
Urea (mg/dl)		68.8	12.5	31.8	7.3	0.000**
Creatinine (mg/dl)		1.4	0.2	0.6	0.2	0.000**
INR		1.6	0.1	1.0	0	0.000**

TLC: total leucocytic count, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, ALT: Alanine aminotransferase, AST: Aspartate transaminase, INR: international normalized ratio.
**P<0.01 highly significant.

Table 2. Number and percentage of *Candida* species isolated from urine and mouth swab in patients with cirrhosis by the VITEK 2 technique.

Urine culture		
***Candida* species**	**Number**	**Percentage**
Candida albicans	6	**33.3**
Candida spherica	5	27.7
Candida tropicalis	4	22.2
Candida parapsilosis	2	11.1
Candida krusei	1	5.5
Mouth swab culture		
***Candida* Species**	**Number**	**Percentage**
Candida albicans	7	**38.8**
Candida spherica	4	22.2
Candida tropicalis	3	16.7
Candida parapsilosis	2	11.1
Candida krusei	1	5.5
Candida famata	1	5.5

95% confidence interval

Table 3. Distribution of mortality among patients with cirrhosis and associated *Candida* species colonization in oral swab and urine samples

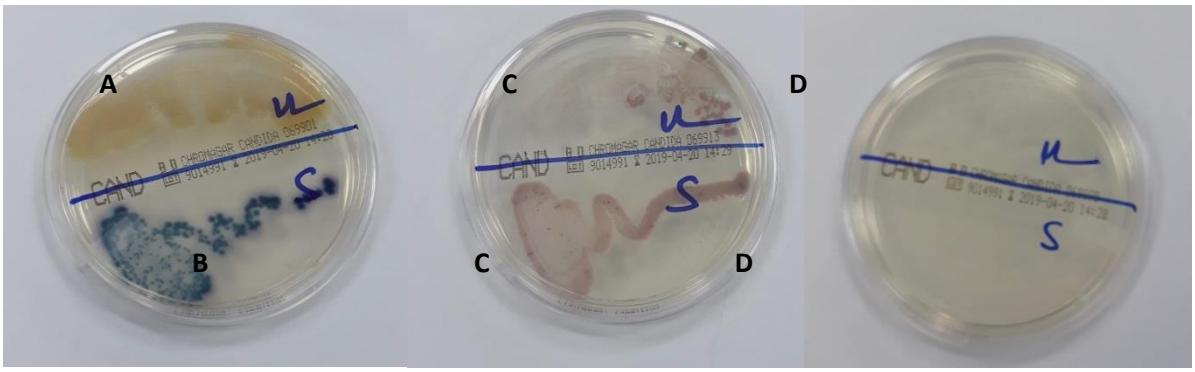
Type of culture	***Candida* species**							
	Candia tropicalis		***Candida albicans***		***Candida krusei***		***Candida parapsilosis***	
	Number of isolates	**Mortality No. %**	**Number of isolates**	**Mortality No. %**	**Number of isolates**	**Mortality No. %**	**Number of isolates**	**Mortality No. %**
Urine culture	16	14 (87.5%)	74	58 (78.4%)	17	12 (70.6%)	3	3 (100%)
Oral swab	14	11 (78.6%)	66	52 (78.8%)	15	12 (80%)	5	4 (80%)

P<0.05 significant

Table 4. Urine samples and oral swabs with mixed *Candida* species

Urine samples Total= 21	***C.tropicalis***	***C.albicans***	***C.krusei***	***C. spherica***	***C.parapsilosis***
9	0	1	1	0	0
7	1	1	0	0	0
3	0	1	0	1	0
1	0	1	0	0	1
1	1	0	0	1	0
Oral swabs Total= 17	***C.tropicalis***	***C.albicans***	***C.krusei***	***C. spherica***	***C.parapsilosis***
7	0	1	1	0	0
6	1	1	0	0	0
1	0	1	0	1	0
2	0	1	0	0	1
1	1	0	0	1	0

Figure 1. Growth of different candida species on CHROM agar™ *Candida* media.

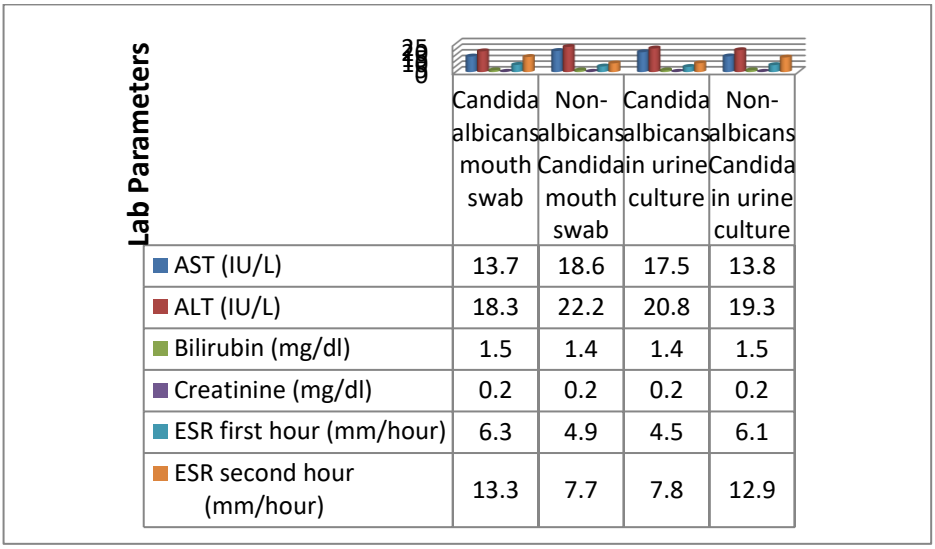


A: *Candida albicans* isolated from urine sample, **B**: *Candida tropicalis* isolated from mouth swab, **C**: *Candida krusei* isolated from both urine and mouth swab cultures,

D: No *Candida* species were detected from both urine and mouth swab cultures.

U: Urine sample, **S**: Mouth swab

Figure 2. Significant laboratory parameters among patients with positive *Candida* species isolated from urine and mouth swab cultures.



Discussion

Cirrhosis and immune dysfunction have a bidirectional relationship [10]. Fungal infections lead to a rise in the risk of mortality at any liver disease stage [11]. Hence, early detection and accurate identification are very essential for effective therapeutic outcomes [12].

In this study, *Candida albicans* was the most prevalent fungal infection in both urine (74%) and mouth swab cultures (66%) in cirrhotic patients. This goes with the study made by Kamani and Kalwar who stated that *Candida albicans* species were the most common fungal pathogen in urine culture in patients suffering from liver cirrhosis and was observed in 70 of 141 (49.6%) patients, followed by *non-albicans Candida* species in 30 (21.3%) patients [13]. None of the controls showed colonization of *Candida* in either urine or mouth specimens. Cirrhosis in our study had increased risk of having *Candida albicans* compared to controls. This highlights the effect of decompensated liver disease on immune system with the resultant cirrhosis-associated immune dysfunction which is associated with substantial mortality risk [14].

By applying VITEK 2 technique in our study, the most prevalent *Candida* species detected

was *Candida albicans* in both urine and mouth swab cultures. This finding is in concordance with the study done by Kaur *et al.* ,who evaluated the VITEK 2 technique for clinical identification of *Candida* species in hospitalized patients and found that *Candida albicans* (82.51%) was the most commonly isolated and that VITEK 2 technique is an alternative method for identification and sensitivity testing of *Candida* species [15].Concerning antifungal resistance, this study stated a higher resistance to fluconazole among *Candida albicans* (50%) compared to other *Candida* species. However, no resistance for voriconazole was noted in *Candida albicans* or other *Candida* species in urine cultures. This agrees with the study done by Berkow and Lockhart who found that several genes and mutations increase resistance to fluconazole in clinical isolates, primarily in *C. albicans*. They recommended that in order to preserve the utility of fluconazole, it is important to fully appreciate the manner by which all *Candida* species exhibit resistance to it [16].This highlights the importance of performing sensitivity testing on fungal cultures to avoid using resistant medications in critically ill patients in the ICU minimizing their chance of survival.

This study showed a higher resistance among *non-albicans Candida* isolated from both urine and mouth swab to caspofungin compared to *Candida albicans* isolated from mouth swab only and this agrees with the study performed by Coste *et al.,* that found echinocandin resistance among *Candida* species specially *Candida glabrata* [17]. Although caspofungins are the safest antifungals in cirrhosis, other lines of therapy should be considered with the lack of clinical response especially in the setting of *non-Candida* infections as fluocytosine, polyenes and allylamines [18].

In this study, the highest mortality rate among both urine cultures and mouth swabs was found to be due to *Candida parapsilosis* and this agrees with the study made by Hirano *et al.,* who studied the mortality rate of candidemia in hospitalized patients and found that among patients less than 65 years, *Candida parapsilosis* was the most often isolated species [19].

Conclusion

According to this study, cirrhotic patients admitted to the intensive care unit had a 3–4 times higher risk of infection than the control group due to the high prevalence of *Candida albicans.* Among cirrhotic patients admitted to the intensive care unit, *Candida* species infection is linked to elevated death rates. Multiple antifungal medication resistance is present in *Candida* species detected in cirrhotic patients admitted to the intensive care unit. In order to improve survival for patients in critical condition, voriconazole might be taken into consideration. It is highly effective against *Candida* species with limited resistance. Tight monitoring of severely ill cirrhotic patients admitted to intensive care units in order to detect and treat fungal infections early and improve survival rates.

Funding statement:

This research received no specific grants from any funding agency.

Competing interests

None declared.

References

1- Agvald-Ohman C, Klingspor L, Hjelmqvist H and Edlund C. Invasive candidiasis in long term patients at a multidisciplinary intensive care unit: Candida colonization index, risk factors, treatment and outcome. Scand. J. Infect. 2008; 40:145-153 Doi: 10.1080/00365540701534509

2- Alam MZ, Alam Q, Jiman-Fatani A, Kamal MA, Abuzenadah AM, Chaudhary AG et al. Candida identification: a journey from conventional to molecular methods in medical mycology. World J Microbiol Biotechnol. 2014; 30(5):1437-51. doi:10.1007/s11274-013-1574-z.

3- Izquierdo A, Melhem A, Bonfietti LX and Rodriguez-Tudela JL. Susceptibility test for fungi: Clinical and laboratorial correlations in medical. Revista Do Instituto de Medicina Tropical de Sao Paulo. 2015; 57: 57-64. doi: 10.1590/S0036-46652015000700011.

4- Sampaio P and Pais C. Epidemiology of Invasive Candidiasis and Challenges for the Mycology Laboratory: Specificities of Candida glabrata. Curr Clin Micro Rpt 1. 2014: 1–9. Doi:10.1007/s40588-014-0002-y

5- Shrestha R, Gyawali N, Gurung R, Amatya R. and Bhattacharya SK. Effect of Urogenital Cleaning with Paper Soap on Bacterial Contamination Rate While Collecting Midstream Urine Specimens. J Lab Physicians. 2013; 5(1): 17–20. doi: 10.4103/0974-2727.115910.

6- World health organization: How to safely collect oral swabs (saliva) from deceased patients suspected to be infected with Ebola or Marburg, 2017.

7- Carroll K C, Pfaller MA, Landry ML, McAdam AJ, Patel R, Richter SS, et al. Manual of clinical microbiology, 12th ed. American Society for Microbiology, Washington, DC. 2019.

8- Ganeshkumar A, Nagarajan P, Mahalingam P, Balasubramanian S, Archunan PA, Govindaraju A, et al. Antifungal susceptibility and virulence profile of Candida isolates from abnormal vaginal discharge of women from southern India. Eur J Obstet Gynecol Reprod Biol. 2020; 254: 153-158. Doi: 10.1016/j.ejogrb.2020.09.0.

9- Clinical and Laboratory Standards Institute: Performance standards for antifungal susceptibility testing of yeasts. CLSI Supplement M60. Wayne, PA.2017.

10- Tuchendler E, Tuchendler PK, Madej G. Immunodeficiency caused by cirrhosis. Clin Exp Hepatol. 2018; 4: 158-164. doi: 10.5114/ceh.2018.78119.

11- D'Amico G, Morabito A, D'Amico M, Pasta L, Malizia G, Rebora P, et al.: Clinical states of cirrhosis and competing risks. J. Hepatol. 2018; 68: 563–76. doi:10.1016/j.jhep2017.10.020.

12- Deurukhkar SC, Saini S. laboratory approach for diagnosis of candidiasis through ages. Int. J. Curr. Microbiol. App. Sci 3, 2014: 206-218.

13- Kamani L, Kalwar H. Fungal urinary tract infection among chronic liver disease patients with hepatic encephalopathy and its treatment outcomes. JGH Open, 2021; 5: 213-218 doi:10.1002/jgh3.12470.

14- Tandon P, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. Semin Liver Dis. 2008; 28: 26-42. Doi: 10.1055/s-2008-1040319.

15- Kaur R, Dhakad MS, Goyal R, Haque A, Mukhopadhyay G. Identification and Antifungal susceptibility testing of Candida species: A Comparison of Vitek-2 system with conventional and molecular methods. J Global Infect Dis 2016; 8:139-46. Doi: 10.4103/0974-777X.192969.

16- Berkow EL, Lockhart SR. Fluconazole resistance in Candida species: a current perspective. Infect Drug Resist. 2017; 10: 237–245. Doi: 10.2147/IDR.S118892.

17- Coste AT, Kritikos A, Li J, Khanna N, Goldenberger D, Garzoni C, et al.: Emerging echinocandin resistant Candida albicans and glabrata in Switzerland. Infection 48. 2020; 761-766. Doi: 10.1007/s15010-020-01475-8

18- Yeoh SF, Lee TJ, Chew KL, Lin S, Yeo D, Setia S. Echinocandins for management of invasive candidiasis in patients with liver disease and liver transplantation. Infect Drug Resist. 2018; 11: 805–819. Doi: 10.2147/IDR.S165676.

19- Hirano R, Sakamoto Y, Kudo K, Ohnishi M. Retrospective analysis of mortality and Candida isolates of 75 patients with candidemia: a single hospital experience. Infect Drug Resist 2015; 8: 199-205 Doi: 10.2147/IDR.S80677.

Hasan E, El Serafy M, Eletreby R, Shousha H, Wassef M, Salem S, Anwar R, Mostafa N, Elawady B. Prevalence of *Candida* species colonization in patients with chronic liver disease admitted to the intensive care unit: A prospective two center study. Microbes Infect Dis 2025; 6(1): 389-395.