Original Article

Evaluation of candidemia risk factors and *Candida* species distribution in intensive care units among patients with and without COVID-19

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ABSTRACT

الأهداف: تقييم متغيرات الخطر المتعلقة بأنواع المبيضات في الدم لكل مريض تم إدخاله إلى وحدة العناية المركزة بغض النظر عن المريض مع أو بدون تشخيص كامل لـCOVID-19، خلال الفترة من مارس 2019م إلى ديسمبر 2022م.

المنهجية: مقارنة تقييم البيانات الديموغرافية والسريرية للمرضى المصابين بفيروس كورونا (COVID-19) الإيجابي والسلبي المصابين بفطريات الدم المؤكدة في الدم، أجرينا تقييم 113 حالة. المتغيرات مثل الجنس، والعمر، وعمر الاستشفاء، وتاريخ الاستشفاء، والعدوى المتزامنة، وعلم وظائف الأعضاء الحاد وتقييم الصحة المزمنة - درجات II، وفحص الاعتلال المشترك، والتنبيب، واستخدام القسطرة الوريدية المركزية، واستخدام التغذية الوريدية، واستخدام الستيرويد، واستخدام المضادات الحيوية، ونقص الغدد الليمفاوية، ومن ثم تقييم المتغيرات المختبرية. قمنا بتحديد توزيع أنواع المبيضات والحساسية المضادة للفطريات في مزرعة الدم.

النتائج: كان مرض فيروس كورونا 19 موجودًا في 62.8% من الحالات المؤكدة لمرض كوفيد-19، وكانت هذه الحالات مختلفة بشكل كبير عن الحالات السلبية لكوفيد-19. وجدنا اختلاف مهم إحصائياً في مزيد من التنبيب، واستخدام القسطرة الوريدية المركزية، والتغذية الوريدية، والعلاج الستيرويد في المجموعة الثانية. ولم تكن هناك أهمية مع توزيع الأنواع والعدوى المرتبطة بها. بشكل عام، كان لدى المصابين بـCOVID-19 مستويات أعلى من الهيموجلوبين، وناقلة أمين الأسبارتات، وناقلة أمين الألانين، وخلايا الدم البيضاء، والتي قد ترتبط بإمكانية الكشف عن المبيضات في الدم والسيطرة عليها.

الخلاصة: المبيضات البيضاء والمبيضات الشاذة (*C. parapsilosis*) هي الأنواع التي تظهر في مرضى كوفيد- 19 المصابين، بينما توجد المبيضات البارابسيلوسيس والمبيضات الاستوائية في المرضى غير المصابين بكوفيد- 19. وكانت عوامل الخطر هي التنبيب، والتغذية الوريدية، والقسطرة الوريدية المركزية، والستيرويد في مجموعة كوفيد- 19.

Objectives: To assess the risk variables related to the types of candidemia for each patient, who was admitted into the intensive care unit regardless of the patient with or without complete diagnosis of COVID-19, during the period of March 2019 to December 2022.

Methods: The evaluation comparison of demographic and clinical data of COVID-19 positive and negative patients with candidemia confirmed in blood, 113 cases were assessed. Variables such as gender, age, age of hospitalization, history of hospitalization, concurrently infection, The acute physiology and chronic health evaluation-II scores, comorbidity checking, intubation, central venous catheter use, parenteral nutrition use, steroid use, antibiotic use, lymphopenia, and laboratory variables were evaluated. *Candida* species distribution, antifungal susceptibility in blood culture were determined.

Results: Coronavirus disease-19 was present in 62.8% of cases confirmed candidemia, and these cases were significantly different from COVID-19 negative cases. Significance was found in more intubation, central venous catheter use, parenteral nutrition, and steroid therapy in Group 2. There was no significance with species distribution and associated infection. In total, COVID-19 positive had higher hemoglobin, aspartate aminotransferase, alanine transaminase, and white blood cell levels, which may be associated with the possibility of revealing and controlling candidemia.

Conclusion: *Candida albicans* and *Candida Parapsilosis* (*C. parapsilosis*) are the species seen in infected COVID-19 patients, while C. parapsilosis and *Candida tropicalis* are found in non-COVID-19 ones. Risk factors were intubation, parenteral nutrition, central venous catheter, and steroid in the COVID-19 group.

Keywords: COVID-19, candidemia, risk factors, intensive care unit, cross infection

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When pneumonia cases detected in Wuhan city, China, in December 2019, they were associated with the novel pathogen 'severe acute respiratory syndrome coronavirus-2' on March 11, 2020, the World Health Organization declared a global pandemic. Hospital admissions increased, especially for pneumonia and organ failure cases. Consequently, healthcare systems and governments globally redoubled efforts to accelerate the fight against the virus. As a result of the increase in cases, intensive care units (ICUs) saw an increase in admissions globally as medical systems prepared to handle the growing public health emergency.[1,2]

Candida infections are made more likely by the use of antibiotics, invasive medical procedures, the injection of steroids, and subsequent bacterial infections. These elements impair human immunity, upset the balance of microbiological flora, and facilitate the colonization and proliferation of *Candida* species. There is a higher chance of invasive *Candida* infections, such as candidemia, in those receiving these medicines or dealing with these ailments. In patient populations who are vulnerable, careful monitoring and preventive interventions are essential to minimizing these potentially fatal consequences. Identifying the etiological agents of secondary infections in patients monitored in the ICUs is crucial for empirical antimicrobial treatment selection.[3] Coronavirus disease-19 infection often leads to acute respiratory distress syndrome, a severe respiratory condition with inflammatory exudation and damage to the airways. This can also result in a decrease in CD4+T and CD8+T cell counts, enhancing immunosuppression and compromising immune defenses. These changes increase the risk of fungal infections, posing additional challenges in clinical management.[4]

Among the number of hospital units, ICUs are characterized by the highest antibiotic usage and antibiotic resistance. Several factors account for this tendency. They include extended durations of hospitalization, catastrophic diseases of patients, extensive background (namely, diabetes mellitus, renal and hypertension ones, and more) and utilization of invasive operations for more critical disease control in the form of mechanical ventilation, as well as urinary, nasogastric, peripheral, and central venous catheters (CVCs).[5]

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Healthcare-associated infections (HAIs) are complex diseases that are impacted by a number of factors in healthcare environments. *Candida* species are the most prevalent microorganisms linked to HAIs, ranking third to sixth in prevalence rankings, following *Staphylococcus aureus*, *Acinetobacter spp.*, *Pseudomonas aeruginosa*, and *Enterococcus spp*. *Candida* species can cause major clinical problems and have a high mortality rate, which makes them worrying when identified in relatively small amounts. The high fatality rates linked to these infections highlight the clinical importance and public health risk that *Candida*-associated HAIs offer.[6-11]

Severely ill COVID-19 patients under ICU observation face an elevated susceptibility to candidemia, primarily attributable to exposure to an array of risk factors. Candidemia, prevalent among this patient cohort, tends to manifest within the intricate milieu of early diagnosis and treatment complexities characteristic of hospital ICUs, thereby assuming critical importance as a bloodstream infection with considerable mortality rates. The utilization of broad-spectrum antibacterial medications, administration of parenteral nutrition, implementation of invasive medical procedures, prolonged neutropenia, and the presence of immunosuppressive states collectively contribute significantly to the heightened vulnerability of severe COVID-19 patients to candidal infections.[12]

Candida albicans (*C. albicans*), the primary *Candida* species in candidemia treatment, is facing a shift towards non-albicans species like *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei*, which are emerging as significant pathogens in various therapeutic settings, challenging its traditional dominance in managing candidiasis. This shift may be due to the increased use of broad-spectrum antibiotics, an increase in immunocompromised individuals, and the distinct antifungal resistance profiles of these non-albicans species. The changing distribution of *Candida* species is crucial for guiding clinical practice and enhancing patient care strategies, as they differ from *C. albicans* in their antifungal susceptibility profiles and clinical outcomes.[13]

The specific mechanisms by which COVID-19 infection increases susceptibility to candidemia, the impact of COVID-19-induced immunosuppression on *Candida* infections, and the extent to which the shift towards non-albicans *Candida* species affects treatment outcomes and patient care strategies are still unknown. This study aimed to explore the risk factors, clinical characteristics, and *Candida* species distribution in ICUs during the COVID-19 pandemic. It aimed

to answer questions regarding the differences in candidemia development between COVID-19 positive and negative patients, the impact of COVID-19 on candidemia, the distribution of *Candida* species causing infections, and the correlation between laboratory parameters and candidemia in COVID-19 positive patients. The findings also aimed to provide insights into the manifestation and management of candidemia in ICUs, particularly in the context of COVID-19.

Methods. A retrospective analysis of the literature was carried out at Kütahya University of Health Sciences, Faculty of Medicine, Kütahya, Turkey, from March 2019 to December 2022.

A total of 113 cases were included in the study. The inclusion criteria were set for patients aged 18 and over diagnosed with healthcare-associated candidemia. Upon not meeting the above requirements, the patient was excluded from the study.

Depending on the COVID-19 test, the healthcare-associated candidemi patients were divided into 2 groups: members from Group 1 who tested nosopharyngeal COVID-19 swab test positive for COVID-19 versus Group 2, who tested negative.

This study was approved by Kütahya University of Health Sciences, Faculty of Medicine, Kütahya, Turkey, rectorate ethical principles and ethics committee with the decision numbered 2023/103217 and E-41997688-050.99-103217 at the meeting dated August 16, 2023 which was carried out in accordance with the Declaration of Helsinki.

Retrospective data were gathered from hospital information system and medical records for patients with healthcare-associated candidemia who were being monitored alongside COVID-19 in ICUs which was carried out in Kütahya University of Health Sciences, Faculty of Medicine Hospital, Kütahya, Turkey, started in August 2023. We analyzed demographic characteristics, including age and gender, comorbid diseases such as diabetes mellitus (DM), immunosuppression, malignancy, chronic kidney failure, cardiovascular diseases, and the length of stay at the hospital, history of antibiotic usage, the acute physiology and chronic health evaluation-II scoring, and additional interventions such as foley catheter implementation, catheterization, and mechanical ventilation. We also had other aspects to consider, such as the *Candida* species' type in the blood cultures, typing, antifungal sensitivity, co-infections with Gram-positive or Gram-negative agents, C-reactive protein (CRP), procalcitonin, white blood cells (WBC) and hemoglobin in the blood, the biochemical test results, and antimicrobial treatment.

Conventional techniques and an automated Phoenix system (Becton Dickinson, USA) were utilized for the identification of *Candida* species and their subsequent typing at the species level. Antifungal sensitivities were determined using the Sensititre™ Yeast One system (Thermo Fisher Diagnostic, USA), employing the colorimetric minimum inhibitory concentration measurement method. Bacterial identifications were carried out using conventional methods and the automated Phoenix system (Becton Dickinson, USA).

The standards used in the evaluation of antifungal susceptibility test results are summarized in **Appendix 1 & 2.**

The Clinical and Laboratory Standards Institute antifungal susceptibilities M27 (CLSI-M27) standards were used in the evaluation of antifungal tests.

For *Candida glabrata*, The Food and Drug Administration criteria were used according to the kit recommendation (**Appendix 1**).

For *Candida spp* and *Candida kefyr*, breakpoints in the CLSI-M27 standards were used. This is the rubric in the kit (**Appendix 2**).

Complete blood counts were analyzed using Mindray BC-6800 automated hematology analyzer (Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) with original reagents. Serum aspartate aminotransferase (AST), alanine transaminase (ALT) activities and creatinine, and C-reactive protein (CRP) levels were measured on Beckman Coulter AU5800 analyzer (Beckman Coulter, Miami, FL, USA) with original reagents. Serum procalsitonin levels were measured on Maglumi 2000 analyzer (SNIBE, Shenzhen, China) with original reagents.

The study used the Mindray BC-6800 automatic hematology analyzer for complete blood counts, Beckman Coulter AU5800 clinical chemistry analyzer for creatinine and CRP levels, and Maglumi 2000 chemiluminescence immunoassay analyzer for serum pro-calcitonin concentrations. The original reagents provided by the manufacturers were used for all measurements, ensuring precision and accuracy in the laboratory results.

Statistical analysis. The data were analyzed by The Statistical Package for the Social Sciences, version 16.0 (SPSS Co., Chicago, IL, USA) for Windows. Numerical data were tested for normality using the Kolmogorov-Smirnov test. Normally distributed data were expressed as mean ± standard deviation (SD) and not normally distributed data were expressed as the median and interquartile range (IQR). The categorical variables were expressed as numbers and percentages (%). The comparisons of numerical variables between groups

were tested using the independent-sample t-test or Mann-Whitney-U test according to the distribution of data. The comparisons of categorical variables between groups were tested using the Chi-square (χ^2) or Fisher's exact tests. Statistical significance was established at the level of <0.05.

The traditional cutoff point of *p*-values less than 0.05 was utilized in the current study in order to provide statistical significance, reject the null hypothesis, and determine the existence of significant differences or correlation in data.

Results. The study found that COVID-19 was present in 62.8% of 113 confirmed candidemia cases, with a majority of females (47.9%) and males (52.1%). There were no significant variances in either group's age or gender from the other, which comprised patients without COVID-19. Instead, the 2 groups' age and gender distributions were similar.

Significant differences between groups were found in hospital stay (p=0.0002), intubation status (p=0.008), parenteral nourishment use (p=0.009), CVC's presence (p=0.002), and steroid use (p<0.0001). This means that clinical practice and outcomes were significantly different between the groups this study compared.

Group 2 had a higher number of patients with intubation, CVC, parenteral nutrition, and steroid treatment during hospitalization compared to with Group 1.

The evaluation of the associated infections was carried out through the patient's clinical signs, laboratory results, and test cultures obtained from the patient's blood, tracheal aspirate, as well as urine. The most frequent isolates in Group 1 were Gram-positive and Gram-negative. There were 15/6 (21.1/14.3%) Gram-positive and 36/25 (50.7/49.3%) Gram-negative isolates.

Bloodstream infections related to other factors accompanying candidemia were more common in Group 1 (n=29/22).

Demographic data and accompanying isolates related to candida growth are presented in Table 1.

The primary *Candida* species responsible for candidemia varied between COVID-19 and non-COVID-19 groups. In the COVID-19 group, *Candida albicans* (53.5%) and *Candida parapsilosis* (19.7%) were the predominant species, while in the non-COVID-19 group, *Candida parapsilosis* (59.5%) and *Candida tropicalis* (11.9%) were in the lead.

The distribution of Candida species is presented in Table 2. The distribution of *Candida* species exhibited discernible variations between the studied groups (p=0.0001, χ^2=29.87, df=7). The distribution of antifungal susceptibility for both groups is presented in Table 3. In the COVID-19 positive group, the most sensitive antifungal was anidulofungin (n=70, 98.5%), while in the other group, 41 (97.6%) individuals showed similar sensitivity to all echinocandins (anidulofungin, caspofungin, and micafungin).

The susceptibilities of the 2 groups to antifungals were not significantly different from each other (p=0.600, χ^2=25.51, df=28). The fluconazole was found to be susceptible to 50 (70.4%) of the COVID-19 positive group and 35.7% of the COVID-19 negative group. While the antifungal sensitivity rate was higher in the COVID-19 positive group, sensitivity to all echinocandins was not statistically significantly different between groups. Table 4 shows the distribution of antifungal sensitivities among COVID-positives and COVID-negatives. Patients and the various *Candida* species and the patients' sensitivity to the entities which included anidulafungin, caspofungin, voriconazole, micafungin, and fluconazole. Group 1 was made up of COVID-19 positives while 2 included COVID-19 negatives. The table provided a comparison between Group 1 versus Group 2, indicating that there was no significant difference between Group 1 and Group 2 on antifungal susceptibility patterns.

The distribution of infectious agents accompanying candidemia is presented in Table 5. Gram-positive bacteria were isolated in 15 (21.1%) individuals in the COVID-19 positive group and 6 (14.3%) individuals in the negative group, showing a significant difference between the 2 groups in terms of Gram-positive agents (p=0.024, χ^2=16.10, df=7). In the COVID-19 group, *Enterococcus faecium* (n=8, 53.3%) and Coagulase-Negative *Staphylococci* (CNS; 33.3%) were the most prevalent, while in the COVID-19 negative group, CNS was the most frequently observed.

The distribution of Gram-negative infectious agents accompanying candidemia presented in Table 5. Gram-negative bacteria were identified in 36 (50.7%) individuals in the COVID-19 positive group and 25 (59.5%) individuals in the negative group. No significant difference was observed between the groups in terms of Gram-negative bacteria (P=0.131, χ^2=11.18, df=7).

The evaluation of laboratory tests is shown in Table 6. Significant differences were found between the 2 groups for hemoglobin (p=0.03), AST (p=0.0003), ALT (p=0.04), and WBC (p=0.0002). It was discovered that COVID-19 positive patients had greater levels of WBC, hemoglobin, AST, and ALT than COVID-19 negative patients. On the other hand, there were no

Table 1 - Comparisons of the demographic and clinical data between coronavirus disease-19 positive and coronavirus disease-19 negative patients.

Variables	COVID-19 (+)	COVID-19 (-)	*P*-values	χ^2	df
Gender					
Female Male	34 (47.9) 37 (52.1)	20 (47.6) 22 (52.4)	0.978	0.001	1
Age (years), mean±SD	71±15	68±17	0.402		
Number of days of hospitalization, median (IQR)	29 (20-40)	46 (28-85)	0.0002*		
Concomitant Gram positive infection					
Yes No	15 (21.1) 56 (78.9)	6 (14.3) 36 (85.7)	0.366	0.82	1
Concomitant Gram negative infection					
Yes No	36 (50.7) 35 (49.3)	25 (59.5) 17 (40.5)	0.363	0.83	1
Other cultures					
Blood Urine Tracheal aspirate The others	29 (59.6) 3 (5.9) 16 (31.4) 3 (5.9)	22 (71.0) 1 (3.2) 7 (22.6) 1 (3.2)	0.636	1.71	3
APACHE 2 score, mean±SD	18.5±8.5	21.7±11.8	0.101		
Expected mortality (%), median (IQR)	25.0 (12.0-55.0)	31.4 (20.3-67.1)	0.074		
Comorbidity					
Yes No	59 (83.1) 12 (16.9)	29 (69.0) 13 (31.0)	0.082	3.02	1
Intubation					
Yes No	54 (76.1) 17 (23.9)	40 (95.2) 2 (4.8)	0.008*	6.94	1
Intubation day after hospitalization, median (IQR)	3 (1-10)	2 (1-6)	0.655		
CVC					
Yes No	57 (80.3) 14 (19.7)	41 (97.6) 1 (2.4)	0.009*	6.89	1
Parenteral nutrition					
Yes No	53 (74.6) 18 (25.4)	41 (97.6) 1 (2.4)	0.002*	9.96	1
Steroid					
Yes No	62 (87.3) 9 (12.7)	12 (28.6) 30 (71.4)	<0.0001*	40.30	1
Antibiotics					
Yes No	63 (88.7) 8 (11.3)	41 (97.6) 1 (2.4)	0.092	2.84	1
Lymphopenia					
Yes No	42 (59.2) 29 (40.8)	19 (45.2) 23 (54.8)	0.151	2.06	1

Values are presented as numbers and percentages (%), mean ± standard deviation (SD), or median and interquartile range (IQRs). The comparisons of numerical variables between groups were analyzed with the independent-sample t-test and the Mann-Whitney-U test according to the distribution of data. The comparisons of categorical variables between groups were analyzed with the Chi-square (χ^2) test. *A *p*-value of < 0.05 was considered significant. COVID-19: coronavirus disease-19, CVC: central venous catheterization, APACHE: acute physiology and chronic health evaluation, χ^2: Ki-kare, df: degree of freedom

appreciable variations found in the groups' levels of procalcitonin, creatinine, CRP, or platelet count.

Discussion. In the context of HAIs, candidemia is a significant cause of mortality, and certain risk factors generally impact its occurrence. Nevertheless, our analysis revealed no appreciable differences in the progression of candidemia between age and gender groups. This indicates that within the study cohort, susceptibility to candidemia was not significantly influenced by either gender or age. Invasive procedures in ICUs are crucial risk factors for HAIs, particularly for bloodstream infections like candidemia. The use of CVCs, catheter location and application method, and

Table 2 - Distribution of *Candida* species isolated from coronavirus disease-19 positive and coronavirus disease-19 negative patients.

Candida species	COVID-19 (+)	COVID-19 (-)	*P*-value	χ^2	df
C. parapsilosis	14 (19.7)	25 (59.5)			
C. glabrata	5 (7.0)	4 (9.5)			
C. albicans	38 (53.5)	4 (9.5)			
C. tropicalis	4 (5.6)	5 (11.9)			
C. spp	7 (9.9)	1 (2.4)	0.0001*	29.87	7
C. kefyr	1(1.4)	1 (2.4)			
C. krusei	1 (1.4)	1 (2.4)			
C. lusitaniae	1 (1.4)	1 (2.4)			
Total	71 (100)	42 (100)			

Values are presented as numbers and percentages (%). The comparisons of categorical variables between groups were analyzed with the Chi-square (χ^2) test. *A *p*-value of <0.05 was considered significant. COVID-19: coronavirus disease-19, χ^2: Ki-kare, df: degree of freedom, C.: *Candida*

Table 3 - Distribution of susceptible antifungals in coronavirus disease-19 positive and coronavirus disease-19 negative patients.

Antifungal	COVID-19 (+)	COVID-19 (-)	*P*-value	χ^2	df
Anidulafungin	70 (98.5)	41 (97.6)			
Caspofungin	68 (95.7)	41 (97.6)			
Vorikonazol	60 (84.5)	23 (54.7)	0.164	6.51	4
Micafungin	69 (97.1)	41 (97.6)			
Flukonazol	50 (70.4)	15 (35.7)			

Values are presented as numbers and percentages (%). COVID-19: coronavirus disease-19, χ^2: Ki-kare, df: degree of freedom

Table 4 - Distribution of antifungal susceptibilities of *Candida* species isolated from coronavirus disease-19 positive (group 1) and coronavirus disease-19 negative (group 2) patients.

Candida species	Anidulafungin		Caspofungin		Vorikonazol		Micafungin		Flukonazol
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1- Group 2
C. parapsilosis	13 (18.6)	25 (61)	12 (17.6)	25 (61)	10 (16.7)	14 (60.9)	13 (18.8)	25 (61)	2 (4.0) - 4 (22.2)
C. glabrata	5 (7.1)	4 (9.8)	5 (7.4)	4 (9.8)	3 (5)	2 (8.7)	5 (7.2)	4 (9.8)	3 (6.0) - 3 (16.6)
C. albicans	38 (54.3)	4 (9.8)	37 (54.4)	4 (9.8)	35 (58.3)	4 (17.4)	37 (53.6)	4 (9.8)	35 (70.0) - 4 (22.2)
C. tropicalis	4 (5.7)	4 (9.8)	4 (5.9)	4 (9.8)	2 (3.3)	2 (8.7)	4 (5.8)	4 (9.8)	4 (8.0) - 3 (16.6)
C. spp	7 (10)	1 (2.4)	7 (10.3)	1 (2.4)	7 (11.7)	0 (0.0)	7 (10.1)	1 (2.4)	6 (12.0) - 2 (11.1)
C. kefyr	1 (1.4)	1 (2.4)	1 (1.5)	1 (2.4)	1 (1.7)	0 (0.0)	1 (1.4)	1 (2.4)	0 (1.0) - 1(5.5)
C. krusei	1 (1.4)	1 (2.4)	1 (1.5)	1 (2.4)	1 (1.7)	1 (4.3)	1 (1.4)	1 (2.4)	0 (1.0) - 1(5.5)
C. lusitaniae	1 (1.4)	1 (2.4)	1 (1.5)	1 (2.4)	1 (1.7)	0 (0.0)	1 (1.4)	1 (2.4)	0 (0.0) - 0(0.0)
Total	70 (100)	41 (100)	68 (100)	41 (100)	60 (100)	23 (100)	69 (100)	41 (100)	50 (100) - 18 (100)

Values are presented as numbers and percentages (%). P-value of 0.600, χ^2=25.51, df=28. The comparisons of categorical variables between groups were analyzed with the Chi-square (χ^2) test (a *p*-value of <0.05 was considered significant). C.: Candida, χ^2: Ki-kare, df: degree of freedom

the duration of catheterization, as well as hospital and ICU length of stay, are essential factors.

There was a statistically significant difference between groups in terms of the length of hospital and ICU stays, intubation, CVCs usage, parenteral nutrition, and steroid administration as risk factors for candidemia. The results of our research establish that patients in COVID-19 negative group stayed longer in the hospital while patients which turned out to be COVID-19 positive were treated with lower

Table 5 - Distribution of Gram (+) and Gram (-) bacterial species accompanying *Candida* infection isolated from coronavirus disease-19 positive and coronavirus disease-19 negative patients.

Gram bacteria species	COVID-19 (+)	COVID-19 (-)	*P*-value	χ^2	df
Gram (+) bacteria species					
Coagulase-negative Staphylococci	5 (33.3)	4 (66.6)	0.024*	16.10	7
Enterococcus faecium	8 (53.3)	0 (0.0)			
Enterococcus spp	2 (13.3)	2 (33.3)			
Total	15 (100)	6 (100)			
Gram (-) bacteria species					
Acinetobacter spp	13 (36.1)	7 (28)	0.131	11.18	7
Klebsiella spp	15 (41.7)	10 (40)			
Escherichia coli	2 (5.6)	0 (0.0)			
Pseudomonas spp	2 (5.6)	6 (24)			
Stenotrophomonas maltophilia	1 (2.8)	0 (0.0)			
Proteus spp	0 (0.0)	2 (8)			
Klebsiella spp + Pseudomonas	1 (2.8)	0 (0.0)			
Klebsiella spp + Acinetobacter	2 (5.6)	0 (0.0)			
Total	36 (100)	25 (100)			

Values are presented as numbers and percentages (%). The comparisons of categorical variables between groups were analyzed with the Chi-square (χ^2) test. A *p*-value of <0.05 was considered significant.
COVID-19: coronavirus disease-19, χ^2: Ki-kare, df: degree of freedom

Table 6 - Comparisons of the laboratory data between coronavirus disease-19 positive and coronavirus disease-19 negative patients.

Variables	Group 1	Group 2	*P*-values
WBC (10/uL)	14.16 (7.92-17.89)	8.45 (6.09-17.28)	0.03*
HGB (g/dL)	9.6 (8.6-11.7)	8.7 (8.0-9.4)	0.0003*
PLT (10/uL)	168.000 (86.000-277.000	153.000 (94.750-449.000)	0.593
Procalcitonin (ng/mL)	1.49 (0.46-4.58)	2.21 (0.79-5.32)	0.206
CRP (mg/L), mean±SD	155.2±79.2	163.5±99.2	0.625
Creatinine (mg/dL)	0.97 (0.70-1.83)	0.90 (0.69-1.90)	0.816
AST (U/L)	30 (28-79)	30.5 (18-64.5)	0.04*
ALT (U/L)	32 (28-72)	16 (10.3-53.3)	0.0002*

Normally distributed data are presented as mean ± standard deviation (SD) and not normally distributed data were expressed as median and interquartile range (IQRs). The comparisons of data between groups were analyzed with the independent-sample t-test and the Mann-Whitney-U test according to the distribution of data. *A *p*-value of <0.05 was considered significant. WBC: white blood cell, HGB: hemoglobin, PLT: platelet, CRP: C-reactive protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase, Group 1: coronavirus disease positive, Group 2: coronavirus disease negative

hospitalization period. In contrast, a greater proportion of COVID-19 positive patients accessed the use of steroids, parenteral nourishment, CVCs, and the level of intubation during their time in the hospital were more. These findings may imply differences in patients with and without COVID-19 infection in the severity of their ailment and in the manner they decided to deal with it.

Some of the risks factors associated with the infection were prolonged hospital stay, severe clinical conditions, comorbidities such as DM, chronic kidney disease, hypertension, invasive procedures such as mechanical ventilation, urinary catheterization, CVCs, nasogastric and peripheral venous catheterization, surgical interventions, poor hand hygiene, and infection control measures non-compliance.[5]

As for the distribution of candidemia causative *Candida* species, *Candida albicans*, *Candida parapsilosis* were more detected among vulnerable COVID-19 patients, whereas *Candida parapsilosis* and *Candida tropicalis* were more reported for COVID-19 negative. Anidulofungin was found to be the most commonly sensitive antifungal for COVID-19 patients with an average susceptibility of 98.5%. No significant susceptibility differences among echinocandins; all exhibited an equal 97.6% susceptibility averages among COVID-19-negative cases. Susceptibility to fluconazole in the sensitive group of COVID-19 patients (70.4%),

was higher than for COVID-19 negative (35.7%). Thus, no variability in fluconazole susceptibility between studied groups was found.

Our study showed that *Candida albicans* (n=42) was the predominant causative agent in all patients, followed by *Candida parapsilosis* (n=39). According to Vena et al's study,[13] bloodstream infections are more frequently linked to other *Candida* species, including *Candida parapsilosis*, *Candida tropicalis*, and *Candida glabrata*. This emphasizes taking into consideration of a wider range of *Candida* species than only *Candida albicans* when diagnosing and treating patients with bloodstream infections because different species may differ in terms of pathogenicity, susceptibility to antifungals, and clinical outcomes.

Various studies have investigated the distribution of *Candida* species causing candidemia, revealing distinct patterns. Tigen et al[14] identified *Candida albicans* as the most prevalent species (n=27; 75%), followed by *Candida glabrata* (n=4; 11%), and *Candida tropicalis* (n=3; 8%). Koçak et al[15] reported *Candida albicans* prevalent (55.2%), with *Candida parapsilosis* as the second most common species (28.9%), highlighting the association of CVCs usage and prolonged hospitalization as risk factors for candidemia.

Gültekin et al[16] reported the species distribution in 119 candidemia samples as *C. albicans* (49%), *C. parapsilosis* (23%), and *C. tropicalis* (14%). On the other hand, Öztürk et al[17] identified *C. albicans* (53%), *C. parapsilosis* (30%), and *C. glabrata* (5.5%) as the most common causes of candidemia in 36 samples. In terms of antifungal susceptibility, all isolates showed 100% sensitivity to amphotericin-B. However, resistance was observed in 8% of *C. albicans* to voriconazole and 20% to fluconazole. Itraconazole resistance was observed in all *C. glabrata* and *C. krusei* isolates, while one *C. tropicalis* isolate (50%) and 58% of *C. albicans* isolates exhibited resistance.[17]

In a study spanning 11 years in the ICU carried out by Yang et al,[18] involving 186 patients diagnosed with healthcare-associated fungal infections (516 samples), the most common isolates were *C. albicans* (27.3%), followed by *C. tropicalis* and *C. glabrata* (6.6%) and similarly, Sasso et al[19] reported findings from a 10-year study on 3557 non-urinary culture samples in the ICU, identifying *C. albicans* as the predominant species (57.1%), followed by *C. glabrata* (14.9%) and *C. tropicalis* (9%).

A study on COVID-19 positive patients revealed a 14.4% prevalence of systemic *Candida* infection, with *C. albicans* and *C. parapsilosis* similar to our study's findings.[20] In our study, Gram-positive infections accompanying candidemia were observed in 15 (21.1%) COVID-19 positive and 6 (14.3%) negative cases, while Gram-negative infections were detected in 36 (50.7%) COVID-19 positive and 25 (59.5%) COVID-19 negative cases.

Fluconazole resistance is common in *C. glabrata* and therefore echinocandins are often used as first-line therapy. The presence of resistance to echinocandin therapy has been associated with FKS1 and FKS2 gene alterations.[21] This study found no significant difference in antifungal susceptibility between COVID-19 positive and COVID-19 negative groups. Fluconazole sensitivity was 50% and COVID-19 sensitivity was 35.7% of COVID-19 positives. However, sensitivity to all echinocandins was not statistically different between groups.

Analyzing Gram-positive and negative infections accompanying candidemia based on culture samples, blood culture positivity was present in 29 (59.6%) patients in Group 1 and 22 (71%) in Group 2.

While bacterial or viral coinfections are rarely observed during the diagnosis of COVID-19, an increase in the duration of hospital stay and the need for ICU admission, along with invasive procedures, leads to an elevated incidence of secondary infections.[22,23]

Among HAIs agents, *Candida* species are significant and rank 3-6 after microorganisms such as *Staphylococcus aureus*, *Acinetobacter spp.*, *Pseudomonas aeruginosa*, *Enterococcus spp*. are known to cause high mortality rates.[6-11] In a study investigating co-infections and antimicrobial resistance in the COVID-19 positive group, bacterial/fungal co-infections were identified in 15 (1.4%) cases out of 1093 and among these, 10 (55.6%) were Gram-negative, 2 (11.1%) were Gram-positive bacteria, and 6 were *Candida* species. The isolated agents identified as *Aeromonas hydrophila/caviae* (n=2), *Burkholderia cepacia* (n=2), *P. aeruginosa* (n=2), *Acinetobacter baumannii* (n=2), *S. aureus* (n=2), *C. albicans* (n=2), *C. glabrata* (n=2), and *C. kefyr* (n=2).[23] Prior to COVID-19, 163 (11.9%) out of 1374 blood culture samples showed growth, with 35.0% being Gram-negative, 60.7% Gram-positive bacteria, and 4.3% yeast. During the pandemic, 148 (17.5%) out of 847 blood culture samples exhibited growth, with 31.8% being Gram-negative, 58.1% Gram-positive bacteria, and 10.1% yeast. The frequencies of Gram-positive and Gram-negative bacteria were similar before and after the pandemic (p>0.05).[24] In the same study, both before (40.5%) and during the pandemic (50.7%), CNS were the most frequently isolated bacteria in ICUs, with a significant increase observed during the pandemic. Methicillin resistance in CNS isolates has been shown in studies to range from 40-90.7%.[23,26-28]

Coagulase-Negative *Staphylococci* is among the most commonly isolated bacteria from blood cultures. However, due to their presence in the skin and mucous membrane flora, inappropriate culture methods, and difficulties in distinguishing between colonization and infection, CNS has not been considered an infection agent for a long time. Nevertheless, recent evidence has drawn attention to CNS as a significant cause of HAIs.[28,29] The most frequently reported CNS species include *Staphylococcus hominis* (43.6%), *Staphylococcus epidermidis* (26.3%), and *Staphylococcus haemolyticus* (14.8%).[23]

During the one year study period, a sum of 1859 samples from ICUs patients' obtained samples were analyzed to ascertain the distribution of the causal agents and the sensitivity of the isolates to the various antibiotics. Samples analyzed includes 414 urine, 1085 blood, 210 tracheal aspirates, 69 CSF sample, 29 catheter, 23 wound, 20 sputum, and 9 from sterile body fluid. A total number of 565 isolates, 276 are Gram-positive bacteria, 243 Gram negative bacteria and 46 fungus were harvested among the causal agents. Coagulase-Negative *Staphylococci* accounted for 194 isolates, the most common pathogens found. *Acinetobacter baumannii* followed with 56 isolates, *Escherichia coli* followed with 53 isolates, *Candida* species *S. aureus* followed with 46. These findings emphasize the importance of both bacterial and fungal pathogens in clinical settings, as well as the varied microbiological etiology of candidemia.[9]

Study limitations. The study provides valuable insights into candidemia risk factors. However, it has limitations such as a small sample size of 113 ICU patients, potential recall bias, and a retrospective design. The study's timeframe may not fully capture candidemia prevalence, and selection bias may exist. Variability in clinical practices across different institutions, potential confounding factors, and demographic details also impact the study's robustness.

Future research should address these constraints and improve understanding of candidemia risk factors in COVID-19 infected patients.

In conclusin, *Candida* species, which are microbiocenosis of the human body, becomes pathogenic for the host in each case. Risk factors that predispose to the development include immunosuppression, existing concomitant diseases, invasive diagnostic and medical procedures. *Candida* species even occupy the third to sixth places in a scale of pathogen ranking according HAIs. In patients with COVID-19, central venous catheterization, administration of corticosteroids and parenteral nutrition are independent risk factors for candidemia. The most common *Candida* species in patients who have tested positive for COVID-19 are *C. albicans* and *C.parapsilosis*, and in patients who have tested negative are *C. parapsilosis* and *C. tropicalis*. Early diagnosis, determination of the spectrum of pathogens and their antibiotic susceptibility plays an important role in proper antibacterial and antifungal therapy.

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Appendix 1 - Clinical and Laboratory Standards Institute M27.

Antifungal agents	Susceptible	Susceptible dose dependent	Intermediate	Resistant
Fluconazole*	≤8	16-32		≥64
Itraconzole	≤0.125	0.25-0.5		≥1
Flucytosine	≤4		8-16	≥32
Caspofungin	≤2*†			
Voriconazole	<1			>4

**Candida krusei* recovered from super infections have not been found to be susceptible to fluconazole and may require alternative antifungal therapy.
† In clinical studies, voriconazole MIC_{90} for *C. glabrata* baseline was 4 ug/ml; 13/50 (26%). *C. glabrata* baseline isolates were resistant (MIC ≥4ug/ml) to voriconazole. However, based on 1054 isolates tested in surveillance studies the MIC_{90} was 1 ug/ml.

Appendix 2 - Minimum inhibitory concentration interpretative criteria for *Candida* species as per Clinical and Laboratory Standards Institute M27.

Antifungal agents	Susceptible	Susceptible dose dependent	Intermediate	Resistant	Non-susceptible
Anidulafungin	≤2				>2
Caspofungin	≤2				>2
Fluconazole*	≤8	16-32		≥64	
5-Flucytosine	≤4		8-16	≥32	
Itraconzole	≤0.125	0.25-0.5		≥1	
Micafungin	≤2				>2
Voriconazole	≤1	2		≥4	