



Antifungal susceptibility and speciation of *Candida* isolated from blood at a tertiary care centre

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Abstract. There has been a rise in the incidence and prevalence of fungal infections worldwide, especially by *Candida* spp. leading to significant morbidity and mortality. Early recognition of *Candida* bloodstream infection has been associated with improved outcome in patient care. Hence, the present study was carried out to determine the distribution of the *Candida* species that causes candidemia as well as its antifungal susceptibility pattern in the hospital. A total of 8,087 blood cultures received from various clinical departments of a tertiary care centre were processed via the Automated blood culture system BACTEC FX40 or manually as per standard protocol in the Department of Microbiology from January to December 2022. Isolated *Candida* spp. were identified using biochemical tests and CHROM agar. Antifungal susceptibility was performed and interpreted as per Clinical and Laboratory Standards Institute guidelines. A total of 2,010 blood cultures showed a positive culture growth of microorganisms, out of which, *Candida* spp. was isolated in 123 blood cultures (6.11%). The Neonatal Intensive Care Unit accounted for the isolation of 78.8% of *Candida* spp. *C. krusei* was found to be the most common isolate 36.5% followed by *C. albicans* (21.2%), *C. glabrata* (19.5%), *C. parapsilosis* (13.8%) and *C. tropicalis* (9%). Voriconazole was found to be the most effective antifungal agent, with 81.3% of *Candida* spp. showing susceptibility to it, and was found to be the most effective antifungal agent. Non-albicans *Candida* spp., *C. krusei* was found to be the predominant isolate in the present study. The neonatal age group was the most commonly affected age group in candidemia. It is advisable to monitor the changing trend of *Candida* species in particular, geographical

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area to get an idea about prevalent species and their antifungal susceptibility pattern for choosing empirical therapy and better patient management

Keywords: candidemia; *Candida non-albicans*; antifungal drug sensitivity; voriconazole

Introduction

Fungi have emerged as a major public health concern since the late 20th century and have significantly increased the morbidity and mortality of patients admitted in intensive care units (ICU) and among immunocompromised patients [1]. Increased incidence of candidemia over the years is largely due to advancements in medical interventions, numerous elderly and susceptible population having transplantation and haematological malignancies [2]. According to data from the Centers for Disease Control (CDC), *Candida albicans* is the eighth most prevalent nosocomial infection [1]. As mentioned in the study, the National Nosocomial Infection Surveillance (NNIS) system of the United States, has stated *Candida* as the fourth common pathogen causing nosocomial bloodstream infections (BSI). The international EPIC II study, as quoted by M. Schroeder *et al.* [3], has shown *Candida* to be the third most common source of infection in over 14,000 patients; patients with *Candida* bloodstream infection had a significantly greater mortality rate than those with Gram-positive or Gram-negative bloodstream infections.

Even though *Candida albicans* is thought to be the most common cause of candidemia, since the 1980s, there has been an upsurge in infections brought on by other *Candida* species [3-5]. Since Non-*albicans Candida* (NAC) species frequently exhibit intrinsic and/or acquired resistance to widely used antifungal medications, the advent of NAC is a serious concern. Antifungal medication is now widely used as a preventative measure in high-risk patients suspected of having invasive and systemic candidiasis. Acquired antifungal resistance in *Candida* varies greatly when it comes to antifungal medicines. Reports of a fluconazole-resistant *C. glabrata* and *C. parapsilosis* outbreak are already available [6]. This justifies the need of the speciation of *Candida* isolates for formulating guidelines for empirical therapy at the local level. B. Dalyan Cilo [7] and M. Carbia *et al.* [8] have reported that the range of agents associated with candidemia differs throughout nations, years, even hospitals within the same nation. Because of this, it's critical to perform surveillance studies on a regular basis to track changes in the aetiology and susceptibility pattern related to the treatment of fungal infections in hospitals.

India is a developing country with more than 1.3 billion population, hot and humid weather in most of the states throughout the year, liberal use of over-the-counter antibiotics and steroid, a large pool of undiagnosed/poorly controlled diabetes and other immunocompromised status, so it becomes necessary to have a baseline data for each region [1]. A review of literature suggests that it is better to have baseline data of the local geographical area for better patient care. As scant data relating to candidemia is available for Central Gujarat, the present study was undertaken

to determine the prevalence of candidemia, species distribution and their antifungal susceptibility pattern as blood isolates was taken up.

Materials and Methods

The study was conducted in Central Gujarat, India, at a tertiary care teaching hospital's Department of Microbiology from January 2022 to December 2022. Throughout this time, the hospital received and processed 8,087 blood culture samples from patients who had been hospitalised to different clinical departments with suspected septicemia or pyrexia of unknown cause via Automated blood culture system BD BACTECFX40 (BD diagnostics, USA) or by conventional manual techniques as per the standard procedures [9]. All the blood culture bottles received in the department with adequate volume and proper labeling of patient demographic details from suspected sepsis, irrespective of age, sex and clinical wards were included in the study. Positive flagged blood culture bottles and/or conventionally processed blood culture bottles were then subcultured on Brain heart infusion agar (Microexpress, A division of Tulip Diagnostics Pvt. Ltd, India) and MacConkey agar (Microexpress, A division of Tulip Diagnostics Pvt. Ltd, India.). The inoculated plates were incubated at 37°C for 24 hours. Conventionally processed blood culture bottles were sub-cultured on alternate day to look for growth and incubated maximum for 7 days at 37°C. Automated blood culture system BD BACTEC FX40 processed blood cultures which flagged green at the end of 5 days of incubation were reported negative. Creamy, smooth, pasty and convex colonies on Brain heart infusion agar were subjected to Gram stain. Gram-positive budding yeast-like cells presumptively identified as *Candida* spp. were further confirmedly performing a Germ tube test, the colour of the colony on CHROM agar (Hi-Medial Pvt.Ltd, India), Slide culture on Corn meal agar (Microexpress, A division of Tulip Diagnostics Pvt. Ltd, India) and sugar fermentation test.

For Germ tube test, 500µL of serum was inoculated with a loopful of *Candida* colonies from Brain heart infusion agar and incubated for 2 hours at 37°C. The presence of germ tube was considered as *Candida albicans* isolate. The absence of germ tube development was considered as *Candida non-albicans* species. For further speciation, the colony was cultured on CHROM agar and a different colour for *Candida* spp. was recorded and interpreted as per the manufacturer's instructions. Slide culture on Corn meal agar (Microexpress, A division of Tulip Diagnostics Pvt.Ltd, India.) was also performed for morphological identification and co-relation. Antifungal susceptibility testing by E-strip was carried out on Muller Hinton Agar (Microexpress, A division of Tulip Diagnostics Pvt.Ltd,

India) with added 2% Glucose and 0.5 µg/mL Methylene blue. Five millilitres of sterile saline were used to suspend four to five colonies that were picked up. After vortexing the suspension, the turbidity was brought down to 0.5 McFarland levels. Using a sterile brush dipped in the suspension, lawn culture was performed after the incubation period of approximately 15 to 20 minutes. Antifungal susceptibility was carried out by E strips (Hi-media Pvt. Ltd., India) for Voriconazole (0.002-32 µg/mL), Fluconazole (0.016-256 µg/mL), Ketoconazole (0.002-32 µg/mL) and Amphotericin B (0.002-32 µg/mL). Disc diffusion susceptibility was tested for Clotrimazole (10 µg) and Nystatin (50 µg). The result was interpreted as susceptible, resistant, intermediate or susceptible dose-dependent per CLSI M27M44S [10] and EUCAST [11] guidelines.

Patients' demographic data, place of admission & the laboratory findings were entered in Microsoft Excel for frequency distribution analysis. The Chi-square test was applied for categorical data and statistical significance was

studied using Statulator, an online calculator for analysis and interpretation of result [12]. p-value of <0.05 was considered as the statistical significance and the association was established for that factor. Only clinical samples received by the laboratory for routine analysis were included in the study, with no direct patient involvement. All the data collected were from patients' requisition forms received along with the samples and LIS software of the hospital. Every method used in research with human participants conforms with ethical guidelines [13, 14].

Results

Of 8,087 blood culture samples received in the Laboratory, 2,010 samples (24.85%) were found to be culture positive for microorganisms. *Candida* spp. were isolated in 123 samples (6.11%) from these positive blood culture samples as shown in Figure 1. The bacterial aetiology was not evaluated for present study analysis. On an average, the blood culture took 48-72 hours to be positive for *Candida* growth.

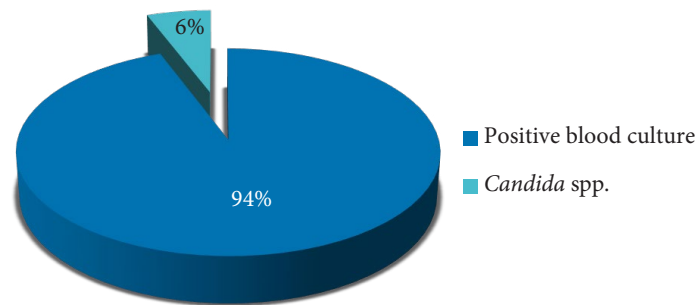


Figure 1. % *Candida* spp. isolated from positive blood culture samples

Source: compiled by the authors

Among the 123 *Candida* isolates, the majority of the *Candida* isolates were from the Neonatal Intensive Care Unit and baby room, being, 97 isolates (78.86%) followed

by the Paediatric ward and Paediatric ICU 13 isolates (10.5%), Medicine wards & ICU had 10 isolates (10.5%) and 3 (1.6%) from others as depicted in Figure 2.

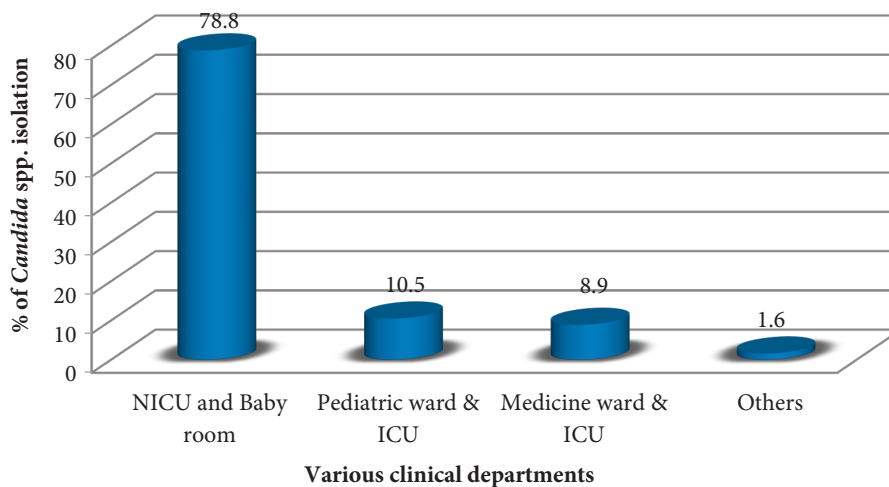


Figure 2. % Distribution of *Candida* isolates from the various clinical departments

Notes: NICU – Neonatal Intensive Care Unit; ICU – Intensive Care Units

Source: compiled by the authors

Analysis of patients' age group-wise distribution showed 102 patients (83%) in <1 year of age group, 7 patients (5.7%) in 1-10 years of age, 11 patients (8.9%) in 30-45 years of age and 3 patients (2.4%) in >60 years of age. Microbiological data analysis was based on the results from culture on CHROM agar, corn meal agar morphology, germ tube and sugar fermentation tests. Based on the different colour production by different species of *Candida* on CHROM agar *Candida* spp. were identified as *C. albicans* (dark green), *C. tropicalis* (dark blue), *C. krusei* (pale pink),

C. parapsilosis (white to pale pink) and *C. glabrata* (white). Chlamydospore formation, arrangements of conidia and/or pseudohyphae on slide culture on corn meal agar confirm the *Candida* speciation. From all the morphological and biochemical analysis, 97 isolates (78.8%) were *Candida* non-albicans and 26 isolates (21.2%) were *C. albicans*. Over all *Candida* species isolated were *C. krusei* (36.5%), *C. albicans* (21.2%), *C. glabrata* (19.5%), *C. parapsilosis* (13.8%) and *C. tropicalis* (9%). The distribution of various *Candida* spp. is shown in Figure 3.

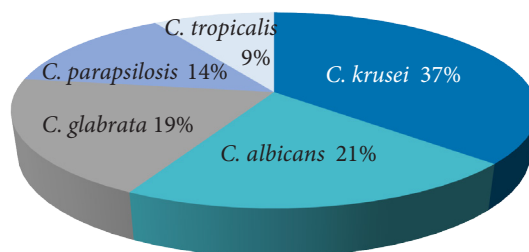


Figure 3. % Distribution of various *Candida* spp.

Source: compiled by the authors

Of the total 123 *Candida* isolates, 84 (68.3%) were from ICU settings and 39 (31.7%) were from ward settings. The speciation of *Candida* isolates from ICU showed a predominance of *Candida* non-albicans, i.e. 66 (78.6%) isolates and 18 (21.4%) as *Candida albicans*. Similarly, 31 (79.5%) were *Candida* non-albicans and 8 (20.5%) were *Candida albicans* in Wards' isolates. The Chi-square test using Statulator, an online calculator, showed a p-value of 0.75 for this comparison, which was not found to be statistically significant. 70 *Candida* isolates (56.9%) were recovered from male patients, while 53

isolates (43.1%) were from female patients. Amongst 70 isolates from men, 56 (80%) were *Candida* non-albicans and 14 (20%) were *Candida albicans*. Of the 53 isolates from women, 41 (77.3%) were *Candida* non-albicans while 12 (22.6%) were *Candida albicans*. The chi-square test using Statulator, an online calculator showed a p-value of 0.81 for this comparison, which is statistically not significant suggesting *Candida* speciation, has no association with the gender of patient. Antifungal susceptibility testing result observed in all *Candida* isolates of the present study is shown in Figure 4.

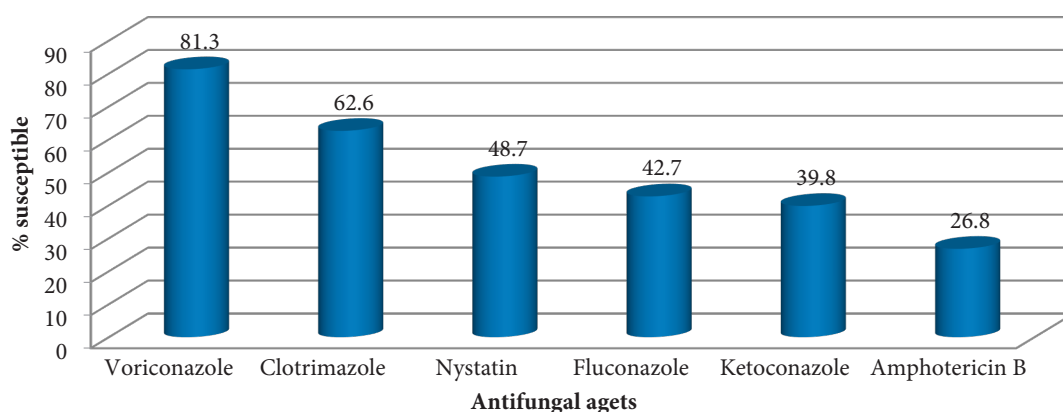


Figure 4. % Susceptibility of all *Candida* isolates to various antifungal agents

Source: compiled by the authors

The overall susceptibility pattern of *Candida* isolates showed 81% susceptibility to Voriconazole followed by 62% Clotrimazole. Least susceptibility was observed to Amphotericin B. Antifungal susceptibility pattern in the *C. krusei* isolates was also evaluated, where 73.3% susceptibility was

observed in Voriconazole followed by 66.66% in Clotrimazole and 28.8% to Nystatin. The high degree of resistance to almost all the azoles suggested intrinsic resistance in the species. Amongst *C. albicans* isolates, the highest susceptibility was observed in Voriconazole (84.6%) followed

by Fluconazole and Clotrimazole (57.6%), Ketoconazole (46.1%), Nystatin (42%) and Amphotericin B (38.4%). The average MIC for Voriconazole ranged from 0.016-0.032 and from 0.50-1 for Fluconazole.

To summarise, the culture positivity rate due to candidemia was 6.11%. A majority of the *Candida* isolates, 78.86% were from Neonatal ICU and baby room. 56.9% of the *Candida* isolates from male patients, with male predominance being observed in the present study. 78.8% isolates were *Candida non-albicans*, *C. krusei* being the most common. Overall, 81.1% isolates were susceptible to Voriconazole. As the data collection was from requisition forms mainly, detailed clinical history of patients, risk factors, co-morbid conditions, average hospital stay, and mortality rate were not evaluated in present study.

Discussion

A common problem for patients admitted to tertiary care facilities is fungal infections. There has been an increase in *Candida* spp. infections over the last 40 years, especially those brought on by *Candida non-albicans* spp. Because of this, the laboratory diagnosis is essential for accurately identifying the species at issue and starting prompt, effective therapy for patients. The prevalence of candidemia in developing countries is reported less because of limited studies in such settings. Even with such limited data, developing countries have reported 4-15 times higher rate of candidemia than developed nation [2].

Table 1 depicts the comparison of the present study with other studies from Indian authors and authors from across the globe.

Table 1. Comparison of present study data with other studies

Study by	Study region	Study duration	Rate of candidemia	Sex predominance	<i>Candida</i> speciation
S. Ahmad <i>et al.</i> [1]	UP, India	2018-2019	-	Male – 53.3%	<i>C.albicans</i> – 28% <i>C.tropicalis</i> – 49% <i>C.parapsiiosis</i> – 10.4%
M. Schroeder <i>et al.</i> [3]	Germany	2008-2017	0.5%	Male – 61.4%	<i>C.albicans</i> – 60.9% <i>C.glabrata</i> – 19.4% <i>C.parapsiiosis</i> – 6.6%
E. Rajni <i>et al.</i> [4]	Rajasthan India	2017-2020	2.8%	M:F=2:1	<i>C.albicans</i> – 11% <i>C.tropicalis</i> – 38% <i>C.parapsiiosis</i> – 18%
E.J. Kim <i>et al.</i> [15]	Korea	2006-2017	2.4%	-	<i>C.albicans</i> – 39.9% <i>C.tropicalis</i> – 20.2% <i>C.parapsiiosis</i> – 18.2%
E. Ghrenassia <i>et al.</i> [16]	France	2002-2017	0.7%	Male – 61%	<i>C.albicans</i> – 54% <i>C.glabrata</i> – 19%
C. Agnelli <i>et al.</i> [17]	Brazil & Spain	2010-2018	-	Male – 57.1%	<i>C.albicans</i> – 45.4% <i>C.parapsiiosis</i> – 20.8% <i>C.glabrata</i> – 14.2%
S. Mazzanti <i>et al.</i> [18]	Italy	2010-2018	2.2%	Male – 62%	<i>C.albicans</i> – 52% <i>C.parapsiiosis</i> – 24% <i>C.glabrata</i> – 14%
N. Alkharashi <i>et al.</i> [19]	Saudi Arabia	2008-2015	-	Male – 53.4%	<i>C.albicans</i> – 33% <i>C.tropicalis</i> – 22.2% <i>C.glabrata</i> – 18.5%
E.E. Ricotta <i>et al.</i> [20]	USA	2009-2017	-	Male – 51%	<i>C.albicans</i> – 48% <i>C.glabrata</i> – 24% <i>C.parapsiiosis</i> – 11%
D. Solomon <i>et al.</i> [21]	Kenya	2019-2020	8.2%	Male – 58%	<i>C.auris</i> – 29% <i>C.albicans</i> – 25.8% <i>C.parapsiiosis</i> – 19.3%
Present study	Central Gujarat, India	2022	6.11%	Male – 56.9%	<i>C.albicans</i> – 21.2%, <i>C.krusei</i> – 36.5%, <i>C.glabrata</i> – 19.5%

Source: compiled by the authors

The higher rate of candidemia in the present study might be due to lesser study duration as compared to the other studies, where they have conducted the studies for 3-15 years. Agnelli *et al.* [17] and N. Alkharashi *et al.* [19] have just mentioned the *Candida* isolates included in their study, so the rate of candidemia was not calculated. A larger study duration gives a better idea about the change in

the rate of candidemia over the years and the prevalence of common species over time. T.S. Al-Musawi *et al.* [22], H. Chawda *et al.* [23] and W. Alkhalifa *et al.* [24] have also reported more cases of candidemia in male patient. All the studies reviewed here show that candidemia is more reported in male patients all over the world. Thus, the present study's finding about male preponderance is well correlated.

78.8% of isolates in the present study were from the NICU and baby room. This finding is similar to the other study from Gujarat by H. Chawda *et al.* [23] who reported 98% isolates from NICU. N. Alkharashi *et al.* [19] have reported 67.6% and E. Rajni *et al.* [4] have reported 78.9% isolates from ICU. S. Sridharan *et al.* [25] have reported that 89.5% candidemia patient in their study had ICU stay. The results of the present study demonstrate that candidemia is a serious problem in intensive care units. This would be linked to advances in organ support systems, overuse of antibiotics, and improved diagnostics, which lengthens ICU stays [1]. The present study area being a tertiary care setting supports this observation.

The most frequent cause of candidemia in this investigation was *Candida non-albicans*, which is consistent with the results of other Indian studies that are included in the table 1 as well as studies by H. Chawda *et al.* [23] and W. Alkhalifa *et al.* [24]. S. Boonsilp *et al.* [26], the authors reported in their study from Thailand that *C. tropicalis* as the most common isolate in candidemia patients. On the contrary, European studies by M. Schroeder *et al.* [3], E. Ghrenassia *et al.* [16] and S. Mazzanti *et al.* [18], found *Candida albicans* predominant isolate. A study by E. Lindberg *et al.* [27] at a Swedish hospital has reported *Candida albicans* as the most common species. N. Alkharashi *et al.* [19] and T.S. Al-Musawi *et al.* [22] from Saudi Arabia also have noted *Candida albicans* as a major isolate.

The most prevalent *Candida non-albicans* isolate in the current investigation was *C. krusei*, which was followed by *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. A study in west Gujarat by H. Chawda *et al.* [23] has reported *C. tropicalis* as the most common species, followed by *C. glabrata*, *C. gullermondii* and *C. parapsilosis*. A North Indian study by H. Kaur *et al.* [2] has observed *C. tropicalis* as the most common species followed by *C. pelliculosa* and *C. krusei*. In a Korean study by E.J. Kim *et al.* [15], the authors have reported *C. tropicalis* to be the most common, *Candida non-albicans* followed by *C. parapsilosis*. These differences point out that different species are predominant in different geographical area, thus making it mandatory for *Candida* speciation for local data.

In the present study, 81.3% susceptibility to Voriconazole was found, followed by 62.6% to Clotrimazole, 48.7% to Nystatin and 42.7% to Fluconazole. N. Alkharashi *et al.* [19] have noted 89% susceptibility to Voriconazole and 60% to Fluconazole in *Candida non-albicans* isolates. S. Ahmad *et al.* [1], in their study from UP, India, have observed susceptibility of 80% to Voriconazole, 67% Fluconazole, 62% to Amphotericin B and 14% to Nystatin. E. Ghrenassia *et*

al. [16] have shown 70% susceptibility to Fluconazole and 92% to Echinocandins. H. Chawda *et al.* [23] have reported 100% sensitivity to Voriconazole, 98.8% to Fluconazole, 73% to Ketoconazole and 68.6% to Clotrimazole. So, if evaluation of the susceptibility data of various antifungal drugs is made, there is a constant increase in the resistance is reported. Therefore, it is important to monitor the change in susceptibility pattern over the years.

Conclusions

At the conclusion of the study, the primary goal of the investigation was to assess the antifungal susceptibility pattern and profile of *Candida* spp. isolated from blood culture in different wards and intensive care units, which was achieved. As per the study result, *Candida non-albicans* was the most common agent causing Candidemia and *C. krusei* was found to be the most common species. The culture positivity rate for candidemia was 6.11%, indicating a significant presence of this infection among the blood culture samples. The majority of *Candida* isolates (78.86%) were from the Neonatal Intensive Care Unit and baby room, indicating a higher vulnerability in this age group. Specifically, 83% of the patients with candidemia were less than 1 year old. Being a tertiary care teaching Hospital catering to critical patients from rural area and adjacent states, ICU setting has a maximum of *Candida* spp. isolation. A male predominance was observed, with 56.9% of the *Candida* isolates being recovered from male patients. Voriconazole was the most effective antifungal drug in the present study area, with more than 80% susceptibility. Therefore, in order to better treat candidemia infections, the current study highlights the importance of routine monitoring investigations in all institutions. This will raise public knowledge of candidemia and its risk factors and provide details regarding the potential long-term effectiveness of community-distributed antifungals. The study identified gaps in data, such as the lack of evaluation of biofilm formation, patient outcomes, risk factors, and detailed clinical histories. Future research should aim to address these aspects to provide a more comprehensive understanding of candidemia and its management. Further studies incorporating these factors will help in formulating more effective treatment protocols and preventive measures.

Acknowledgements

None.

Conflict of Interest

The authors declare no conflict of interest.

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Чутливість до протигрибкових засобів і видова ідентифікація грибків роду *Candida*, ізольованих з крові в третинному медичному центрі

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Анотація. У світі спостерігається зростання захворюваності та поширеності грибкових інфекцій, особливо зумовлених *Candida* spp., що призводить до значної захворюваності та смертності. Раннє виявлення кандидемії було пов'язане з покращенням результатів лікування пацієнтів. Таким чином, дане дослідження було проведено для визначення розподілу видів *Candida*, які викликають кандидемію, а також їхнього профілю чутливості до протигрибкових препаратів у лікарні. Загалом 8,087 гемокультур, отриманих з різних клінічних відділень третинного медичного центру, були оброблені за допомогою автоматизованої системи ВАСТЕС FX40 або вручну за стандартним протоколом у відділенні мікробіології з січня по грудень 2022 року. Виділені *Candida* spp. були ідентифіковані за допомогою біохімічних тестів на хромогенних середовищах. Чутливість до протигрибкових препаратів проводили та інтерпретували відповідно до рекомендацій Інституту клінічних та лабораторних стандартів. Загалом 2,010 гемокультур показали позитивний ріст мікроорганізмів, з яких у 123 культурах (6,11 %) були виділені *Candida* spp. 78,8 % *Candida* spp. були виділені з відділення неонатальної інтенсивної терапії. *C. krusei* була найпоширенішим ізолятом (36,5 %), далі *C. albicans* (21,2 %), *C. glabrata* (19,5 %), *C. parapsilosis* (13,8 %) та *C. tropicalis* (9 %). Серед виділених штамів *Candida* spp. 81,3 % були чутливими до вориконазолу, який виявився найефективнішим протигрибковим препаратом. Серед неальбіканс штамів *Candida* spp., *C. krusei* була найпоширенішим ізолятом у даному дослідженні. Кандидемія найчастіше виявлялася у неонатальній віковій групі. Доцільно відстежувати тенденцію зміни видів *Candida* у конкретному географічному регіоні, щоб отримати уявлення про поширені види та їхній профіль чутливості до протигрибкових препаратів для вибору емпіричної терапії та кращого ведення пацієнтів

Ключові слова: кандидемія; неальбіканс *Candida*; чутливість до протигрибкових препаратів; вориконазол