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PREVALENCE OF CANDIDA SP. IN HOSPITAL TOILETS AND THEIR SENSITIVITY TO COMMERCIAL ANTIFUNGALS

PREVALÊNCIA DE CANDIDA SP. EM SANITÁRIOS DE HOSPITAIS E SUA SENSIBILIDADE AOS ANTIFÚNGICOS COMERCIAIS

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ABSTRACT: **Objective:** To evaluate the prevalence of species of the genus Candida in toilet seats of public and private hospitals in Teresina-PI and to assess their sensitivity profile to antifungals. **Method:** Eight samples were analyzed in duplicates from the surfaces of male and female toilet seats. **Results:** Candida yeasts were detected in 18.75% of the samples, with 6.25% identified as C. krusei and 12.5% as C. glabrata. Both species were significantly inhibited by the antifungals ketoconazole and nystatin, with nystatin exhibiting the largest halo diameter and greater effectiveness. However, variations in variance and standard deviation data were observed. At lower concentrations, fluconazole and nystatin showed reduced inhibition. **Conclusion:** The presence of these species in hospital environments highlights the need for rigorous monitoring and control to prevent fungal infections, contributing to the reduction of morbidity and mortality associated with these infections.

KEYWORDS: Candidiasis. Fungal Infections. Hospital Environments. Infection Control. Sensitivity to Antifungals.

RESUMO: **Objetivo:** Avaliar a prevalência de espécies do gênero Candida em assentos sanitários de hospitais públicos e privados de Teresina-PI e verificar seu perfil de sensibilidade a antifúngicos. **Método:** Foram analisadas 8 amostras, em duplicatas, das superfícies dos assentos sanitários masculinos e femininos. **Resultados:** As leveduras do gênero Candida foram detectadas em 18,75% das amostras, sendo 6,25% identificadas como C. krusei e 12,5% como C. glabrata. Ambas as espécies apresentaram inibição significativa pelos antifúngicos cetoconazol e nistatina, com a nistatina mostrando maior diâmetro de halo e uma ação mais eficaz. No entanto, houve variações nos dados de variância e desvio padrão. Para concentrações menores, a inibição do fluconazol e da nistatina foi reduzida. **Conclusão:** A presença dessas espécies em ambientes hospitalares destaca a necessidade de vigilância e controle rigorosos para prevenir infecções fúngicas, contribuindo para a redução da morbidade e mortalidade associadas a essas infecções.

PALAVRAS-CHAVE: Ambientes Hospitalares. Candidíase. Controle de infecções. Infecções Fúngicas. Sensibilidade a Antifúngicos.

INTRODUCTION

Candida species represent a significant cause of invasive infections in tertiary hospitals, due to their high incidence of 3.9 cases per 1,000 admissions and a lethality rate ranging from 50% to 72%, particularly among immunocompromised and critically ill patients. Although most infections caused by this fungus are of endogenous origin, exogenous acquisition is also possible in healthcare settings through direct contact with colonized or infected patients or after contact with contaminated surfaces. Furthermore, the ability of non-albicans Candida strains to persist for long periods on dry and wet surfaces may increase the likelihood of transmission from the environment.

These microorganisms proliferate and develop rapidly in environments containing organic substrates, considering temperature and relative humidity, making hospital bathrooms an ideal setting for their dissemination. Different species pose a significant threat to patients in Intensive Care Units (ICUs), leading to mortality outcomes and accounting for the most commonly reported healthcare-associated cases. Approximately 11% of infections in American ICUs are caused by Candida.

The apparent increase in the emergence of these species as pathogens can be attributed to improved identification methods, the severity of patients' conditions, the interventions they undergo, and the drugs used. Major risk factors for infections by Candida sp. include prolonged use of broad-spectrum antibiotics, the host's immunocompromised state, and the use of medical devices in surgeries, including catheters.

In recent decades, candidiasis has been associated with Candida species other than Candida albicans. This microorganism accounts for one-third of all vulvovaginitis cases in women of reproductive age, with approximately 8% of women experiencing recurrent episodes. The most common responsible pathogen is the C. albicans species (90%), with most remaining cases caused by Candida glabrata.

When analyzing trends in Candida species in invasive candidiasis from 2009 to 2017 in the United States, as well as other relevant factors at the patient and hospital level, it was observed that 48% of all infections were caused by C. albicans. Next, C. glabrata accounted for 11% of cases and had a higher crude mortality rate than C. albicans, being more frequently detected in patients over 65 years old.

Additionally, biofilms are considered the most efficient growth form of Candida sp. cells and a strong agent in the intensification of antifungal resistance. However, this is associated with the limited availability of antifungal agents, further complicating the selection of the most appropriate treatment option. Moreover, the presence of yeasts colonizing healthcare professionals has raised concerns among hospital infection control teams, as they can serve as potential sources of microorganism contamination through exogenous transmission. Each Candida sp. species has unique characteristics in terms of invasive potential, virulence, and antifungal susceptibility patterns. Non-albicans Candida species have an increased likelihood of resistance to fluconazole, particularly C. glabrata (16%) and Candida krusei (78%).

When investigating the prevalence of Candida species in a university hospital, analyzing cases of colonization and infection, species distribution, risk factors, and antifungal susceptibility, 74% of the 100 samples analyzed corresponded to colonizations and 26% to nosocomial infections. The most frequent species was C. albicans (40%). Non-albicans species accounted for 71.4% of colonization cases and 52.1% of infections. Additionally, C. glabrata exhibited the highest resistance to the tested antifungals, as this species is intrinsically less sensitive.

The daily use of hospital bathrooms can have a significant impact on the spread of infectious diseases, as invasive fungal infections have become an increasingly important cause of healthcare-associated infections (HAIs), causing significant morbidity and mortality in hospitalized and

immunocompromised patients. Furthermore, some studies report resistance against all major classes of antifungal drugs, complicating effective treatments and resulting in rising mortality rates.

With the increase in antifungal-resistant Candida species, understanding the distribution and trends of these species is necessary for physicians to provide appropriate therapy to patients at the onset of the disease. Changes in the epidemiology of fungal infections significantly affect the choice of empirical antifungal therapy, as species exhibit distinct responses to the antifungal used. Recently, there has been an increase in the frequency of antifungal-resistant species, which may be related to the prophylactic use of these drugs in patients at higher risk of developing invasive fungal infections.

Clinical and epidemiological data on hospital fungal infections in Brazil are limited and not comprehensive. Epidemiological studies have revealed changes in the profile of systemic fungal infections caused by Candida species, with non-albicans species emerging as pathogens of concern. This is troubling, as many of these species may exhibit resistance to the most common antifungals. Thus, the present study aimed to evaluate the prevalence of Candida species on toilet seats in public and private hospitals in Teresina-PI and assess their sensitivity profiles to commercial antifungals.

METHODOLOGY

This was a cross-sectional study with a descriptive approach, conducted in 8 (eight) restrooms, both male and female, with 4 (four) in a public hospital and 4 (four) in a private hospital located in Teresina. Samples were collected from the surfaces of toilet seats. The restrooms were freely accessible to patients, staff, and doctors.

All samples were colected during the afternoon shift. The collection was performed using sterile disposable swabs, moistened with 5 ml of sterile saline solution (0.9% NaCl) at the time of collection. The swab was rubbed on the toilet seat surface, then inserted into a sterile test tube containing the saline solution and stored in a cooler at a low temperature.

All isolates were stored in Sabouraud dextrose agar and subcultured for 24 to 48 hours before being inoculated onto chromogenic media, CHROMagarTMCandida, for the identification and differentiation of yeasts based on colony morphology and color. Chromogenic media facilitate the presumptive identification of Candida species and aid in recognizing mixed cultures. The principle is the production of color in colonies through specific enzymatic reactions with a chromogenic substrate in the media. C. albicans, C. tropicalis, C. glabrata, and C. krusei produce green, blue, lilac, and rough pink colonies, respectively, while other species range from white to pink.

Yeast micromorphology was also identified using conventional methods through microculture testing. This technique is based on the principle that incubation in this medium stimulates conidia production and filamentation, allowing the species to be suggested through the study of the presence and arrangement of blastoconidia and pseudohyphae. Sabouraud agar was autoclaved (for 15 minutes at 121°C) and distributed in plates, which were refrigerated until use. For inoculation, Petri dishes containing two slides and two coverslips on a glass holder with a moistened filter paper to create a humid chamber were autoclaved in advance. The medium was cut into cubes approximately 2.0 x 1.5 cm using sterile tweezers and placed on the slides. Colonies were transferred onto a small piece of Sabouraud agar inside the microculture plate (Petri dish, glass slide holder, coverslip, and absorbent cotton) covered with a coverslip. The cotton in the microculture plate was moistened with sterile distilled water to maintain a humid chamber. Microculture plates were kept at 28°C for 48 hours to

visualize structures. Readings were made directly from the prepared slides using 40x and 100x objectives on an optical microscope. After focusing, some slides were photographed.

An antifungal susceptibility test was performed to observe variations in resistance. Three isolates (from the CHROMagarTMCandida culture plate) were diluted in sterile saline solution (0.9% NaCl) and seeded on Petri dishes containing Sabouraud culture medium. Three paper discs impregnated with antifungals—ketoconazole, fluconazole, and nystatin—were added. To improve results, two concentrations were used: 4 µg/ml and 8 µg/ml for each fungal isolate.

A descriptive analysis of the data was conducted, showing absolute and relative frequencies. Amplitude, mean, variance, and standard deviation were also calculated for comparative purposes regarding the resistance profile among the antifungals used in the study, following the research model by Glehn and Rodrigues. Frequency analyses were performed using the SPSS©IBM version 21 software.

RESULTS AND DISCUSSION

In the present study, 16 samples (in duplicates) were collected and analyzed, of which Candida genus yeasts were present in 18.75% of the isolates (3 samples). On Sabouraud agar, growth appeared as homogeneous, shiny, cream-colored colonies, found exclusively in the female restrooms of both private hospitals (Table 1). The isolation of yeast on CHROMagarTMCandida allowed the identification of colonies with morphological characteristics of Candida sp., based on colony morphology and pigmentation, to determine the isolated Candida species. White/lilac colonies and mauve-colored colonies compatible with the Candida glabrata species were observed, as well as light pink, dull colonies with whitish edges. For species confirmation, a microculture test was performed, in which small, round, and uniform blastoconidia characteristic of C. glabrata and pseudohyphae with blastoconidia forming a cross-matchstick appearance characteristic of C. krusei were identified.

Table 1 – Prevalence of Candida spp. on toilet seats in male and female bathrooms of public and private hospitals in the municipality of Teresina-PI, 2024. (N=16)

Most Prevalent Species in Hospitals	Hospitais			
	Public Hospital		Private Hospital	
	Male Bathroom	Female Bathroom	Male Bathroom	Female Bathroom
C. albicans	0%	0%	0%	0%
C. krusei	0%	0%	0%	6,25
C. tropicalis	0%	0%	0%	0%
C. glabrata	0%	0%	0%	12,5%
C. parapsilosis	0%	0%	0%	0%

Source: Own.

Table 2 shows the halos in millimeters obtained on Sabouraud Agar for evaluating resistance variations at concentrations of 4 mg/ml and 8 mg/ml. The results from the experiments represented indicate that the yeasts C. krusei and C. glabrata are primarily affected by the fungistatic action of ketoconazole and nystatin. It was observed that nystatin showed the highest average halo diameter, indicating a greater area of action against the fungus, despite showing variability in variance and standard deviation values. At the lower concentration, less inhibition/resistance was observed for fluconazole and nystatin.

Table 2. Measurements of Candida spp. halos found in toilet seats of male and female bathrooms of public and private hospitals in the municipality of Teresina-PI.

Identified species	Antifungals/ Concentration (mm)					
	Fluconazole		Ketoconazole		Nystatin	
	4µg/ml	8µg/ml	4µg/ml	8µg/ml	4µg/ml	8µg/ml
C. krusei	R	25mm	20mm	R	R	27mm
C. glabrata (A1)	R	R	R	R	R	R
C. glabrata (A2)	R	R	27mm	R	R	26mm
Amplitude	0	25	27	0	0	27
Average	0,0	12,5	18,5	0,0	0,0	20,0
Variance	0,0	208,3	196,3	0,0	0,0	234,3
Standard deviation	0,0	14,4	14,0	0,0	0,0	15,3

A: Resistant; A1: Sample 1; A2: Sample 2.
Source: Own.

Based on the results obtained in this study, which analyzed 16 samples from hospital environments, the presence of yeast species from the genus Candida is evident, with a significant isolation in female bathrooms of private hospitals. The identification of Candida glabrata and Candida krusei species through morphological methods and microcultivation emphasizes the importance of these locations as potential reservoirs for fungal pathogens. The sensitivity tests indicated that both species showed variable resistance to the antifungals tested, with particular emphasis on the more effective fungistatic action of nystatin compared to ketoconazole and fluconazole. This finding aligns with the literature, which addresses yeast resistance to fluconazole, observed through halo formation and statistical data, where the average halo diameters served as a reference for other parameters.

Candida glabrata is a yeast of growing medical relevance, particularly in severely ill patients. It is the second most isolated Candida species associated with invasive candidiasis, only behind C. albicans. Candidemia has been considered a persistent public health problem, with a significant impact on hospital costs and high mortality, and is the seventh most prevalent cause of hospital infections in Brazil. In the United States, candidemia is the fourth most common cause of nosocomial systemic infections, causing 10% of bloodstream infections and 25% of urinary tract infections in ICUs. Among these emerging yeast pathogens, C. krusei is the least studied, despite its high mortality rate (86.4%), especially in patients with neutropenia and hematological malignancies.

Candida species are part of the normal microbiota of the gastrointestinal and genitourinary mucosa in healthy women, although most women experience symptoms of vulvovaginal candidiasis at least once in their lifetime. Colonization of this yeast is influenced by estrogen levels. However, due to the widespread use of hormone replacement therapy, there has been an extended risk period for this alteration. A study conducted with 177 women living in a quilombola community in northeastern Brazil detected the presence of Candida in 28.9% of vaginal fluid samples from quilombola women, with Candida albicans being the most frequent species (49%), followed by Candida krusei (39.2%). It was also observed that women aged 50 years or older had a higher chance of detection.

When evaluating 305 hospitalized patients with heart failure to identify asymptomatic nosocomial candiduria during 2016 and 2017 in a private hospital in northern Iran, a high prevalence of asymptomatic urinary tract infection by Candida was found, with a prevalence rate of 18.8% in individuals over 51 years old and females (70%). In addition to urinary and intravascular catheters, the occurrence of candiduria was significantly related to a history of surgery, diastolic heart failure, and the use of systemic antibiotics. Furthermore, C. glabrata (40.3%) was the most prevalent species, with 85% susceptibility to fluconazole.

The presence of Candida species in urine (candiduria) is a common clinical finding, often representing colonization or contamination by these specimens. However, they can be etiological agents in urinary tract infections, indicators of underlying pathology in the genitourinary system, or even disseminated candidemia. The ability of Candida to form biofilm is crucial to its pathogenicity and persistence on surfaces, especially C. glabrata, which can persist for 4 to 5 months on surfaces.

When evaluating the incidence of Candida albicans and non-albicans species in a Brazilian tertiary hospital from the environment and healthcare professionals, it was observed that the most recurrent non-albicans Candida species was C. glabrata (37.62%), generally considered a low virulence species but with a higher mortality rate than C. krusei (6.93%), similar to the results obtained in this study. All non-albicans Candida species were sensitive to nystatin. Most isolates were sensitive to fluconazole and amphotericin B. As expected, a high rate of resistance to fluconazole was observed in C. glabrata and C. krusei, which are intrinsically less sensitive to this antifungal, similar to our findings.

Fluconazole is the most commonly used azole antifungal due to its low toxicity to the host, high water solubility, and high bioavailability. However, C. krusei has intrinsic resistance to this drug, while it quickly develops acquired resistance to other antifungal drugs. Several studies report resistance against all major classes of antifungal drugs, complicating effective treatments and resulting in rising mortality rates.

When determining the antifungal susceptibility of 187 independent C. glabrata isolates from hospitalized patients in a 1,900-bed tertiary care center in Belgium, a high mortality rate was found, related to both low intrinsic susceptibility and true resistance to fluconazole, identified in a significant proportion of clinical isolates of this fungus. The phenomenon of antifungal resistance in C. glabrata is of fundamental relevance, as the increase in infections caused by this yeast makes therapeutic management difficult due to limited options of antifungal agents available for these types of infections.

The reasons for the increase in non-albicans Candida species are not yet fully understood but may be related to antifungal resistance and the virulence of these microorganisms. Although Candida glabrata is more prevalent in the United States, its occurrence is lower in Latin America. This pattern was observed in other studies, which identified a prevalence of 60.78% for non-albicans Candida. This may be associated with antifungal resistance mechanisms and virulence factors, whether acquired or intrinsic to the fungus, such as the natural resistance of C. krusei to fluconazole.

The main limitation of this study is the local surveillance being restricted to a limited number of public and private bathrooms in Teresina. The collection of bacteria was not correlated with the time or effort dedicated to regular cleaning of each bathroom. Furthermore, it is likely that multiple strains of the same microorganism were present in the sampled locations. The presence of a specific microorganism in bathrooms does not necessarily indicate a risk for users. Although many of the identified strains are pathogenic and may suggest deficiencies in hygiene practices, no correlation was established between our findings and the hygiene practices observed in the public bathrooms. This lack of correlation limits the interpretation of the results and the identification of corrective measures.

The results of this study have important practical implications for the scientific community and clinical practice. Identifying prevalent Candida species and their susceptibility profiles allows for a more targeted approach in selecting antifungal therapies, which could reduce mortality and morbidity rates associated with fungal infections. Furthermore, awareness of the growing resistance of non-albicans species to common antifungals is crucial for the development of prevention and infection control strategies in hospital settings. Antifungal susceptibility testing is essential to guide medical decisions regarding the appropriate medication and dosage, preventing adverse effects due to excessive doses or the selection of resistant strains.

The implementation of monitoring and early diagnosis protocols, along with continuous education for healthcare professionals on best practices for managing fungal infections, can significantly improve the quality of patient care. This study also highlights the need for further research to explore the epidemiology of Candida infections in different contexts, helping to guide public health policies and more effective clinical interventions.

CONCLUSION

The rising incidence of hospital-acquired fungal infections, especially those caused by species of the genus Candida species, poses a major public health challenge. This study contributes to the understanding of prevalence and antifungal susceptibility in hospital environments, highlighting the identification of C. glabrata and C. krusei in toilet seats of hospitals in Teresina-PI. The detection of these yeasts in common-use areas emphasizes the urgent need for infection control and prevention measures. Furthermore, the results regarding the efficacy of ketoconazole and nystatin compared to fluconazole provide valuable insights for selecting appropriate treatments, underscoring the importance of continuous surveillance on antifungal resistance and the management of healthcare-associated infections.

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