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Infecções relacionadas à assistência à saúde causadas por Candida spp. em neonatos críticos: uma análise das superfícies ambientais

Infecciones asociadas a la atención sanitaria causadas por Candida spp. en neonatos críticos: un análisis de las superficies ambientales

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ABSTRACT

Background and Objectives: invasive fungal infections entail high morbidity and mortality rates in Neonatal Intensive Care Units (NICUs) and are accompanied by an increasing prevalence of resistant isolates, highlighting hospital environments as the primary sources of contamination. This study identified *Candida* species in infants in a Brazilian NICU, assessed their clinical and laboratory conditions and characterized the isolates. **Methods:** *Candida* isolates from newborns (NBs) and environments were identified and analyzed for antifungal resistance, virulence factors, and molecular relationships. **Results:** four NBs presented invasive candidiasis, such as *C. albicans* (2 NBs), *C. glabrata* (1 NB), and *C. parapsilosis sensu stricto* (1 NB). All NBs were extremely premature (<29 weeks) and had used at least one invasive device. Two clinical isolates demonstrated resistance, one to fluconazole (*C. parapsilosis sensu stricto*) and the other to micafungin (*C. glabrata*). Five environmental isolates were identified as *C. parapsilosis sensu stricto*, and one of them showed to be fluconazole susceptible-dose dependent. Biofilm was the only virulence factor produced by all nine isolates. Molecular analysis revealed high similarity between one environmental isolate and one clinical isolate of *C. parapsilosis sensu stricto*. **Conclusions:** the results indicated the presence of *Candida* species in infants and NICU environments, with some demonstrating *in vitro* resistance to fluconazole and micafungin. All isolates produced biofilm. A notable genetic similarity was observed between some environmental and clinical isolates, suggesting the environment as a possible source of infection.

Keywords: Invasive Fungal Infections. Cross Infection. Infection Control. Infant Health.

RESUMO

Justificativa e Objetivos: infecções fúngicas invasivas acarretam elevada morbimortalidade em Unidades de Terapia Intensiva Neonatal (UTINs) e estão acompanhadas de um aumento de isolados resistentes, evidenciando o ambiente hospitalar como fonte primordial de contaminação. Este estudo identificou espécies de *Candida* em neonatos em uma UTIN brasileira, avaliou suas condições clínicas e laboratoriais e caracterizou os isolados. **Métodos:** isolados de *Candida* de recém-nascidos (RNs) e do ambiente foram identificados e analisados quanto à resistência antifúngica, fatores de virulência e relação molecular. **Resultados:** quatro RNs apresentaram candidíase invasiva, como *C. albicans* (2 RNs), *C. glabrata* (1 RN) e *C. parapsilosis sensu stricto* (1 RN). Todos RNs eram extremamente prematuros (<29 semanas) e utilizaram algum dispositivo invasivo. Dois isolados clínicos demonstraram resistência, um ao fluconazol (*C. parapsilosis sensu stricto*) e o outro à micafungina (*C. glabrata*). Cinco isolados ambientais foram identificados como *C. parapsilosis sensu stricto*, e um deles mostrou susceptibilidade dependente da dose ao fluconazol. O biofilme foi o único fator de virulência produzido pelos nove isolados. A análise molecular revelou alta similaridade entre um isolado ambiental e um clínico de *C. parapsilosis sensu stricto*. **Conclusões:** os resultados indicaram a presença de espécies de *Candida* em neonatos e no ambiente da UTIN, com algumas demonstrando resistência *in vitro* ao fluconazol e à micafungina. Todos isolados produziram biofilme. Foi observada uma notável similaridade genética entre alguns dos isolados ambientais e clínicos, sugerindo o ambiente como uma possível fonte de infecção.

Descritores: Controle de Infecções. Infecções Fúngicas Invasivas. Infecção Hospitalar. Saúde do Lactente.

RESUMEN

Justificación y Objetivos: las infecciones fúngicas invasivas conllevan altas tasas de morbilidad y mortalidad en las Unidades de Cuidados Intensivos Neonatales (UCINs) y están acompañadas por un aumento en la prevalencia de aislamientos resistentes, destacando el ambiente hospitalario como la principal fuente de contaminación. Este estudio identificó las especies de *Candida* en neonatos en una UCIN brasileña, evaluó sus condiciones clínicas y de laboratorio y caracterizó los aislamientos. **Métodos:** se identificaron y analizaron los aislamientos de *Candida* de recién nacidos (RNs) y del ambiente en relación con la resistencia antifúngica, los factores de virulencia y las relaciones moleculares. **Resultados:** cuatro RNs presentaron candidiasis invasiva, como *C. albicans* (2 RNs), *C. glabrata* (1 RN) y *C. parapsilosis sensu stricto* (1 RN). Todos los RNs eran extremadamente prematuros (<29 semanas) y habían utilizado al menos un dispositivo invasivo. Dos aislamientos clínicos demostraron resistencia, uno al fluconazol (*C. parapsilosis sensu stricto*) y el otro a la micafungina (*C. glabrata*). Cinco aislamientos ambientales se identificaron como *C. parapsilosis sensu stricto*, y uno de ellos mostró susceptibilidad dependiente de la dosis al fluconazol. El biofilm fue el único factor de virulencia producido por los nueve aislamientos. El análisis molecular reveló una alta similitud entre un aislamiento ambiental y uno clínico de *C. parapsilosis sensu stricto*. **Conclusión:** los resultados indicaron la presencia de especies de *Candida* en neonatos y en el ambiente de la UCIN, con algunas mostrando resistencia *in vitro* al fluconazol y a la micafungina. Todos los aislamientos produjeron biofilm. Se observó una notable similitud genética entre algunos aislamientos ambientales y clínicos, lo que sugiere que el ambiente podría ser una posible fuente de infección.

Palabras Clave: Infecciones Fúngicas Invasoras. Infección Hospitalaria. Control de Infecciones. Salud del Lactante.

INTRODUCTION

Neonatal Intensive Care Units (NICUs) are environments where patients are vulnerable to various types of infections. Invasive fungal infections (IFIs) stand out because they contribute to morbidity and mortality, especially in cases of prematurity, low birth weight (LBW) and immaturity of the immune system.¹⁻⁴ In this context, the *Candida* genus emerges as the predominant fungal agent with a high lethality rate ranging from 30-78%.⁵⁻⁷

The incidence of invasive candidiasis (IC) in NICUs ranges between 0.5% and 20%, with *Candida albicans* being the most common species (55-60%).^{1,2,8} The increase in infections by non-*albicans Candida* (NAC) species has been described in the literature, and *C. parapsilosis* is among the most prevalent species that cause candidemia worldwide. In Brazil, *C. parapsilosis* accounts for more

than 20% of *Candida* species isolated in blood cultures.¹ In recent years, the occurrence of fluconazole-resistant *C. parapsilosis* strains has expanded in the workplace around the world, persisting in several hospital niches, resulting in higher mortality rates (50-63.8%).¹

C. parapsilosis causes outbreaks in NICUs, linked to increased morbidity and mortality.^{1,7} The high incidence of these infections suggests failures in hand hygiene among healthcare professionals and in hospital environments.^{3,9} Studies have shown genetic similarity between *C. parapsilosis* isolates from patients and NICU environments,² indicating a common source of infection.⁹

The lack of adequate sanitation, the prolonged presence of fungal species in hospital environments and the ability to produce factors that facilitate infection, evasion of the immune system and adherence to the host surfaces have a major impact on morbidity and mortality

due to *Candida* spp. in NICUs.^{1,5,8} Among the virulence factors, the production of hydrolytic enzymes, such as proteases, lipases, and phospholipases, as well as the formation of biofilms, stands out.^{3,5,10,11}

The present study aims to identify *Candida* species isolated from newborns with bloodstream infections (BSIs) and those isolated in NICU environments. Additionally, we assessed the clinical and laboratory conditions of newborns (NBs) with BSI and characterized the isolates through phenotypic and genotypic tests.

METHODS

Patients and study location

The study was conducted in a NICU of a high-complexity public hospital in southeastern Brazil, which has 20 intensive and intermediate care beds. Infants with laboratory confirmation of IC were included, and demographic and epidemiological data were obtained from medical records. These NBs were monitored daily by the National Healthcare Safety Network (NHSN) epidemiological surveillance system¹² from admission to discharge or death within a period of one year.

Clinical and environmental sample collection and isolate identification

Blood samples were obtained and identified in the hospital's Clinical Analysis Laboratory by traditional methods using the BACT/Alert® system and confirmed by Vitek® systems (bioMérieux–Durham, USA) between March and December 2018.

Samples from NICU environments were collected three times a day, at the beginning of each of the three work shifts, between March and December 2018, according to the protocol described by Menezes *et al.*¹³ The samples were obtained from high-touch surfaces (incubators, monitor tables, respirator monitors, infusion pumps, vital signs monitors, NICU access doors, soap dishes, paper towel holders, tap nozzles, cabinet drawers, light switches, medicine refrigerator doors, medicine preparation tables and bath sink drains).¹⁴

For this purpose, swabs (Plastlabor, Rio de Janeiro, Brazil) pre-moistened with 0.9% sodium chloride were used, which were rubbed vigorously in areas delimited by sterile molds. In the laboratory, the collection material was vortexed, and 0.2 mL of the solution was seeded on plates containing Sabouraud Dextrose Agar (SDA - Isofar, Duque de Caxias, RJ, Brazil) with the addition of chlorphenicol, and on plates with agar chromogenic for *Candida* (Himedia, Mumbai, India). These were incubated at 35°C for up to 72 hours. Fungal isolates were identified using the matrix-assisted laser desorption ionization (MALDI) technique, followed by detection on a time-of-flight (TOF) analyzer, MALDI TOF (Bruker MALDI Biotyper 4.0).

Antifungal resistance profile

The resistance profile of isolates was determined using the broth microdilution technique, as recommended by the Clinical and Laboratory Standards Institute

(CLSI) in documents M27-A3-S3 and M27-S4.^{15,16} The antifungals assessed were fluconazole (Fluoxol, La Paz, Bolivia), amphotericin B (Cristalia, São Paulo, Brazil) and micafungin (Raffo, Buenos Aires, Argentina). The test plates were incubated at 35°C for 24 hours, and the reading was taken using a spectrophotometer with a wavelength of 490 nm. The tests were carried out in duplicate, in independent experiments, and the *C. parapsilosis* ATCC 22019 strain was used as a technique control.

The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of the antifungal that resulted in a 50% reduction in yeast cell growth compared to fluconazole and micafungin, with 90% for amphotericin B.^{17,18} The interpretation of the MIC and the cut-off points for each antifungal followed CLSI documents M59, M60 and M27-S4^{19,20} guidelines as well as the criteria established by Pfaller and Diekema (2012).²¹

Biofilm formation assessment

Biofilm biomass production (0.5% crystal violet) was assessed according to the protocol by Costa-Orlandi *et al.* (2014),²² with modifications. Biofilm metabolic activity (reduction of Aldrich, St. Louis, MO, USA) was carried out using the methodology of Pierce *et al.* (2008).²³ Spectrophotometer readings were at 570 nm for biomass and 490 nm for biofilm metabolic activity.

The results were classified based on the cut-off point of each strain into low, moderate, and high biofilm production for biomass and metabolic activity, following criteria from Marcos-Zambrano *et al.* (2014).²⁴ For biomass, the following optical deviation (OD) was considered: low<0.44; moderate=0.44-1.17; high>1.17. For metabolic activity, the OD considered were low<0.097, moderate=0.097–0.2, high>0.2. Negative controls were wells containing only Roswell Park Memorial Institute (RPMI) broth. The tests were performed in quadruplicate and repeated three times independently.

Extracellular hydrolytic enzyme and hemolytic activity assessment

Assessment of *Candida* spp.'s ability to produce the extracellular hydrolytic enzymes DNase, phospholipase and proteinase, and hemolytic activity followed the protocol by Riceto *et al.* (2015).¹⁰ The tests were performed in duplicate in independent experiments, and the analysis and interpretation of results were carried out as proposed by Menezes *et al.* (2019).²⁵

Molecular analysis

Genetic similarity analysis was performed by random amplified polymorphic DNA (RAPD-PCR), and the extraction of the genomic DNA of isolates was carried out from cultures in SDA medium (24 hours) at 35°C. The primer oligonucleotides used were OPA9 (5'-GGGTAA-CGCC-3'), OPA18 (5'-AGCTGACCGT-3'), OPB11 (5'-GTA-GACCCGT-3') and OPG17 (5'-ACGACCGACA-3') (Operon Technologies Inc.). Reactions and amplification products were conducted according to the protocol established by Riceto *et al.* [2017].²⁶

RESULTS

Sample characterization

During the study period, seven NBs presented BSI, and of these, four were due to *Candida* species, such as *Candida glabrata* (NB 1), *C. albicans* (NB 2 and NB 3) and *C. parapsilosis sensu stricto* (NB 4). All NBs with *Candida* BSI were biological male, extremely premature (<29 weeks) and used at least one invasive device. The average

length of hospital stay was 70 days, and the NB with *C. parapsilosis sensu stricto* infection died (Table 1).

Five isolates were recovered from environmental samples, all identified as *C. parapsilosis sensu stricto*. They were obtained from the surface of an incubator table (sample 2A), from the inside of two incubators (samples 3A and 4A), from a drawer (sample 7A) and from a bench used for preparing medications (sample 8A) (Table 2; Figure 1).

Table 1. Clinical characteristics of newborns with *Candida* spp. bloodstream infection in a Neonatal Intensive Care Unit. Uberlândia, Minas Gerais, Brazil, 2024.

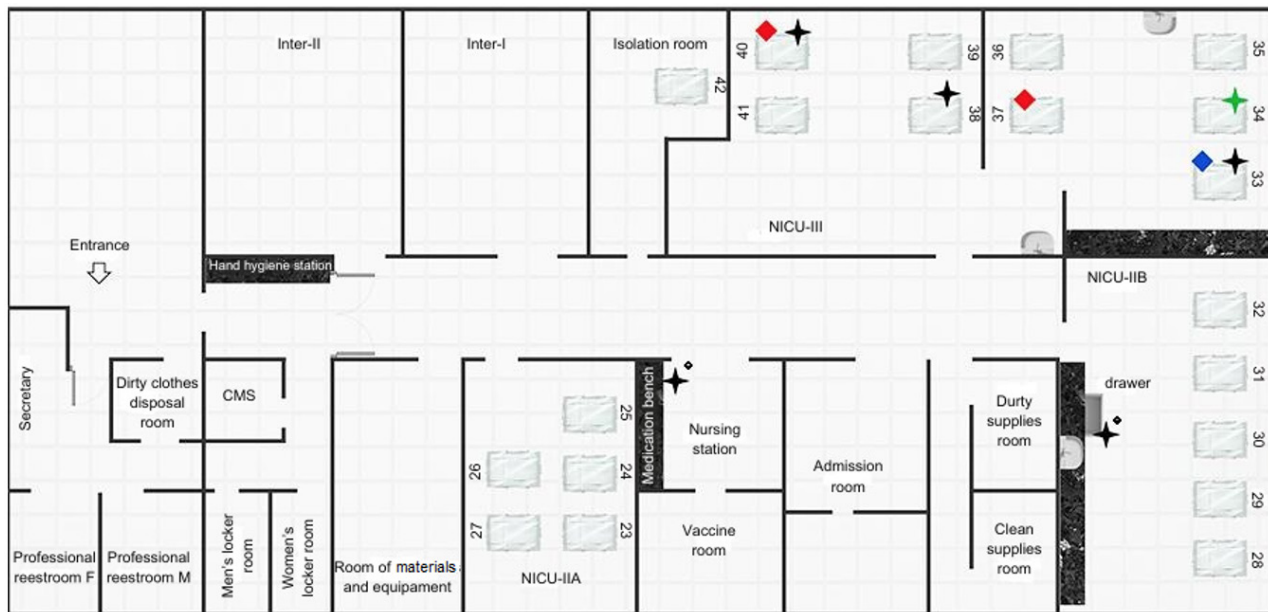
Characteristics	NB 1	NB 2	NB 3	NB 4
Gestational age (weeks)	24.6	24.3	29	25.3
Birth weight (grams)	610	696	1,485	645
Reason for hospitalization	Respiratory distress syndrome; extreme low weight	Respiratory distress syndrome; extreme low weight	Respiratory distress syndrome; extreme low weight	Congenital malformation; extreme low weight
Gastrointestinal tract surgery	NI	NI	NI	EAC
Invasive devices (days of use)				
PICC	35	47	18	15
UVC	3	8	NI	NI
MV	55	77	10	8
PN	23	20	14	12
PVC	14	23	NI	NI
Antifungals (days of use)				
Fluconazole				
Use prior to infection	16	NI	NI	NI
Use after infection	NI	10	22	6
Micafungin				
Use prior to infection	NI	NI	NI	NI
Use after infection	18	NI	NI	6
Length of hospitalization in the NICU (days)	87	126	50	15
Length of hospitalization before BSI (days)	21	5	10	1
BSI date	9/3/18	3/11/18	7/14/18	9/24/18
Outcome	Discharge	Discharge	Discharge	Death

Note: BSI - bloodstream infection; EAC - esophageal atresia correction; NI - no information; NB - newborn; NICU - neonatal intensive care unit; MV - mechanical ventilation; PN - parenteral nutrition; PICC - peripherally inserted central catheter; PVC - peripheral venous catheter; UVC - umbilical venous catheter.

Table 2. Phenotypic characteristics (biofilm production and susceptibility to antifungals) of *Candida* species isolated from environments and bloodstream of infants in the Neonatal Intensive Care Unit. Uberlândia, Minas Gerais, Brazil, 2024.

Species	Local	Collection date	VC	VTT	Amphotericin B MIC (µg/ mL)	Fluconazole MIC (µg/ mL)	Micafungin MIC (µg/ mL)
<i>C. parapsilosis sensu stricto</i> 2A	Surface in incubator table	3/19/18	HBP	HAM	0.50	2.00	1.00
<i>C. parapsilosis sensu stricto</i> 3A	Part internal incubator	3/19/18	MBP	HAM	0.50	1.00	2.00
<i>C. parapsilosis sensu stricto</i> 4A	Part internal incubator	3/19/18	MBP	HAM	0.50	4.00	1.00
<i>C. parapsilosis sensu stricto</i> 7A	Drawer cabinet NICU IIB*	6/26/18	MBP	HAM	0.50	0.50	2.00
<i>C. parapsilosis sensu stricto</i> 8A	Medication bench*	6/26/18	HBP	HAM	0.25	1.00	2.00
<i>C. glabrata</i> (NB 1)	Blood	9/3/18	HBP	HAM	1.00	2.00	2.00
<i>C. albicans</i> (NB 2)	Blood	3/11/18	HBP	HAM	0.50	0.50	0.03
<i>C. albicans</i> (NB 3)	Blood	7/14/18	MBP	HAM	0.50	1.00	0.03
<i>C. parapsilosis sensu stricto</i> (NB 4)	Blood	9/24/18	MBP	HAM	1.00	8.00	2.00

Note: HAM - high activity metabolic; HBP - high biomass production; MBP - moderate biomass production; MIC - Minimum Inhibitory Concentration; NICU - Neonatal Intensive Care Unit; VC - violet crystal; XTT - tetrazole salt; *isolates considered identical by the combined analysis of primers OPA09, OPA18, OPB11 and OPG17.



Note: *C. albicans* 1 and 2 (red diamonds), *C. glabrata* (blue diamond), and *C. parapsilosis sensu stricto* (green star), all of them *u* from the blood culture, and *C. parapsilosis sensu stricto* from the environment culture (black stars); • identical isolates.

Figure 1. Schematic representation of the Neonatal Intensive Care Unit and location of isolation of environmental and clinical samples included in the study. Uberlândia, Minas Gerais, Brazil, 2024.

Antifungal susceptibility test

Two clinical isolates demonstrated resistance to at least one of the antifungals tested: *C. parapsilosis sensu stricto* to fluconazole and *C. glabrata* to micafungin. Furthermore, one environmental isolate (4A) showed to be fluconazole susceptible-dose dependent (SDD) (4µg/mL). Table 2 describes MIC values.

Virulence factor production assessment

All isolates demonstrated the ability to form biofilm *in vitro*, exhibiting high metabolic activity. Furthermore, 44.4% (4/9) were classified as producing biomass at high levels, including two (22.2%) isolates from environments (2A and 8A). The production of extracellular hydrolytic enzymes (DNase, phospholipase and proteinase) or hemolytic activity was not observed in any of the isolates (Table 2).

Isolate genetic similarity determination

Molecular analysis revealed a cluster (A) with five highly similar *C. parapsilosis sensu stricto* isolates ($S_j > 80\%$). This group included four environmental samples (2A, 3A, 7A, 8A) and one clinical sample (NB 4) (Figure 2). Two environmental samples (7A and 8A) were considered identical. All highly similar samples from environments were collected in the first two moments, with an interval of 99 days between the first and second collection. Clinical sample was collected 90 days after the last environmental isolates (7A and 8A) (Table 2).

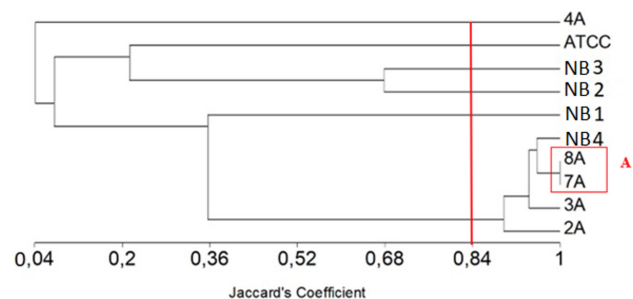


Figure 2. Dendrogram of *Candida* spp. isolates originating from Neonatal Intensive Care Unit environments and the bloodstream of infants with combined analysis of primers OPA09, OPA18, OPB11 and OPG17. Uberlândia, Minas Gerais, Brazil, 2024.

DISCUSSION

IC is often underdiagnosed, with an estimated non-detection rate of between 30% and 70%.²⁷ The lack of epidemiological data in NICU, especially in developing countries, is evident. Although the importance of hospital environments in the spread of microorganisms, including resistant ones, is recognized,²⁷ there are few studies on the presence of pathogenic fungi in this context, espe-

cially in NICUs. However, studies carried out in countries in Latin America, Africa and Asia have demonstrated the presence of *Candida* spp. in NICUs, highlighting it as a potential source of healthcare-associated infections (HAIs).

In this study we identified three *Candida* species causing IC in the four NBs (*C. albicans*, *C. parapsilosis sensu stricto* and *C. glabrata*) and one in NICU environments (*C. parapsilosis sensu stricto*). The relationship between *C. parapsilosis* infections and NICU environments indicates the negative impact on NB survival, especially when inadequate hygiene measures contribute to the transmission of the pathogen microorganisms.^{2,3,9}

Maintaining a clean environment and applying rigorous hand hygiene measures, as well as reinforcing cleaning, disinfection protocols and monitoring the effectiveness of these practices, is crucial to ensuring patient safety, mainly when there are risks of outbreaks caused by resistant pathogens.⁷

In Brazil, some studies have shown a varied distribution of *Candida* species causing IC encompassing BSIs and deep-seated candidiasis, according to different regions of the country.⁵ In the northeast, *C. albicans* (35.3%), *C. tropicalis* (27.4%), *C. parapsilosis* (21.6%) and *C. glabrata* (11.8%) were the most frequent. In the north, *C. albicans* predominated (44%), followed by *C. glabrata* (19%), *C. tropicalis* (19%) and *C. parapsilosis* (14%). In southeastern Brazil, a frequency of 81.1% was reported for *C. parapsilosis sensu stricto*.⁵ This highlights the predominance of NAC species in the country. *Candida* spp. has already been reported in several hospital areas, including the hands of healthcare professionals. Although most *Candida* species infections are endogenous, hospital environments can also be a source, especially in cases of critically ill patients.¹⁴ In our study, *C. parapsilosis sensu stricto* was isolated in NICUs from high-touch surfaces, what is of concern due to the potential increased risk of cross-contamination or nosocomial transmission. The inherent vulnerability of premature NBs due to the immaturity of the immune system and the fragility of epithelial barriers makes them more prone to IC.⁶ All NBs in the study were born at less than 30 weeks of gestation and weighing less than 1,500 grams. Generally, IC manifests itself around the fourth week of life;³ however, in this study, the average time for IC development ranged from 1 to 21 days, with *C. parapsilosis* manifesting more quickly than *C. glabrata*.

The predominance of *C. parapsilosis* can be explained by its colonization in the skin microbiota of healthy individuals and its ability to adhere to surfaces, by the ability to form biofilm (all isolates of this study formed biofilm). A previous study showed *C. parapsilosis* on inanimate surfaces, hands, and infection in the same NICU, and the isolates demonstrated phenotypic and genetic similarities, revealing this microorganism's ability to remain in the unit for months, suggesting infections through cross-transmission or even intestinal translocation, supporting our results.³

C. parapsilosis is prone to colonizing intravascular catheters and proliferating in individuals using parenteral nutrition.^{2,9} The four infants analyzed in this study used peripherally inserted central catheter and received parenteral nutrition therapy. One NB had a congenital malformation in the esophagus and was extremely LBW (645 grams), affected by *C. parapsilosis sensu stricto* infection died six days after diagnosis of candidemia. That strain showed resistance to fluconazole, high metabolism in the biofilm, and demonstrated genetic similarity with environmental samples from NICUs (isolated from the drawer, and from the medication handling bench), despite having differences in resistance to antifungals. This result highlights the complexity of interactions between environmental and clinical strains, and the importance of surveillance and understanding resistance factors.

The *in vitro* resistance in *C. parapsilosis* from IC has been reported in several countries, including Brazil,^{3,7} being associated with the occurrence of outbreaks.⁷ The occurrence of invasive infections by fluconazole-resistant *C. parapsilosis* in NICUs is a significant concern due to the negative impact on patient prognosis and neonatal mortality rates,⁷ considering that fluconazole is the first-choice antifungal for IC treatment in NICUs in different countries. The reduced susceptibility to fluconazole of *C. parapsilosis* isolated from the NICU environment study has been previously reported,^{4,7} and draws attention because it is a unit that cares for critically ill patients and the occurrence of infections due to environmental isolates be something possible. In our study, the isolate, besides showing dose-dependent susceptibility to fluconazole, had a moderate to high capacity for biofilm formation, what is related to protection against antifungal drugs and the immune response, in addition to enabling survival in environments hospital conditions, also resisting the action of disinfectants and desiccation.⁷ Biofilm forming by environmental isolates has been previously related.²

Strategies such as care protocols, efficient management of antimicrobials and hygiene practices are crucial to prevent infections in NICUs.⁷ Given the vulnerability of infants to infections due to the immaturity of the immune system and the frequent use of invasive devices,⁶ this study provides clinical and environmental data on infections by *Candida* in NICUs. Furthermore, the research highlights the scarcity of information on this topic, highlighting the relevance of this study in the epidemiology of HAIs caused by *Candida* spp. and the need for more research in this area.

In conclusion, this study identified *Candida* species in infants and in NICU environments, demonstrating resistance to fluconazole and micafungin, in addition to all isolates forming biofilm. A high genetic similarity was observed between some environmental and clinical isolates, suggesting environments as possible sources of infection. These results are in line with findings in literature, reinforcing the importance of environmental surveillance, rigorous hand hygiene practices and frequent disinfection of hospital environments, especially in high-touch areas, such as surfaces of incubators.

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AUTHORS' CONTRIBUTIONS

Priscila Guerino Vilela Alves contributed to the literature search, writing of the abstract, introduction, methodology, discussion, interpretation and description of results, preparation of tables, conclusions, review and statistics. **Isadora Caixeta da Silveira Ferreira** contributed to the project administration, literature search, writing of the abstract, introduction, methodology, discussion, interpretation and description of results, conclusions, review and statistics. **Ralciane de Paula Menezes** contributed to the writing of the abstract, methodology, interpretation of results, conclusions, review and statistics. **Mário Paulo Amante Penatti** contributed to the writing of the abstract, review and statistics. **Reginaldo dos Santos Pedroso** contributed to the writing of the abstract, review and statistics. **Denise Von Dolinger de Brito Röder** contributed to the project administration, literature search, writing of the abstract, introduction, methodology, discussion, interpretation and description of results, conclusions, review and statistics.

All authors approved the final version to be published and are responsible for all aspects of the work, including ensuring its accuracy and integrity.