UNIVERSIDADE FEDERAL DE UBERLÂNDIA
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IMPACTO DA TRANSMISSÃO CRUZADA DE MICRO-ORGANISMOS EPIDEMIOLOGICAMENTE IMPORTANTES EM UMA UTI NEONATAL

PRISCILA GUERINO VILELA ALVES

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Medicina da Universidade Federal de Uberlândia, como requisito parcial para a obtenção do título de Doutor em Ciências da Saúde.

Área de concentração: Ciências da Saúde.

Orientadora: Prof. Dra. Denise von Dolinger de Brito Röder

Co-orientador: Prof. Dr. Reginaldo dos Santos Pedroso

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Priscila Guerino Vilela Alves

Impacto da transmissão cruzada de micro-organismos epidemiologicamente importantes em uma uti neonatal

Presidente da banca: Profa. Dra. Denise von Dolinger de Brito Röder

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Medicina da Universidade Federal de Uberlândia, como requisito parcial para a obtenção do título de Doutor em Ciências da Saúde.

Área de concentração: Ciências da Saúde.

Banca Examinadora

Titular: Profa. Dra. Erika Nascimento

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Instituição: Universidade de Franca

DEDICATÓRIA

Ao meu filho Augusto que me fez entender o que é o amor verdadeiro e me faz querer ser melhor todos os dias. Te amo filho!

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Ao meu esposo Hitallo, e ao meu filho Augusto;

A minha irmã Patrícia;

Aos meus irmãos Mariana e Murilo;

Aos meus sobrinhos;

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As minhas avós;

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"É justo que muito custe o que muito vale". Santa Teresa D'Ávila

RESUMO

Introdução: Infecção de corrente sanguínea por *Candida* spp. é reconhecida como uma das principais causas de morbidade e mortalidade em recém-nascidos. Estudos realizados em todoo mundo têm mostrado a associação das infecções adquiridas no ambiente hospitalar à prática inadequada de higienização das mãos e à reservatórios inanimados. Esse fato ocasiona a veiculação ambiental de micro-organismos virulentos e a preocupação com a segurança dos RN torna-se ainda maior. Em razão disso, a avaliação epidemiológica das infecções em UTIN se faz necessário. **Objetivo:** analisar espécies de *Candida* isoladas das mãos de profissionais da saúde, antes e após a higienização com gel à base de álcool etílico 70%, do ambiente e de infecção de corrente sanguínea na UTIN, e avaliar os fatores de virulência, produção de biofilme e similaridade genética. **Metodologia:** Os isolados foram identificados utilizando MALDI-TOF. Os testes de susceptibilidade aos antifúngicos foram realizados de acordo com documentos do Clinical and Laboratory Standard Institute. Os isolados foram analisados quanto à produção de enzimas hidrolíticas extracelulares (hemolisina, Dnase, proteinase e fosfolipase) atraves de testes fenotípicos (ágar gema de ovo, ágar albumina bovina, ágar sangue de carneiro 7% e ágar Dnase); a formação de biofilme e atividade metabólica foi avaliada através da coloração com cristal violeta e XTT, respectivamente. A similaridade genética foi realizada através da metodologia DNA polimórfico amplificado aleatoriamente (RAPD-PCR). A vigilância epidemiológica foi realizada atráves do sistema "National Healthcare Safety Network". **Resultados:** *C. parapsilosis* complex foi a espécie mais frequente. Três isolados obtidos das mãos dos profissionais foram resistentes à anfotericina B, um foi resistente à micafungina e cinco foram resistentes ao fluconazol. Um isolado de ambiente apresentou susceptibilidade dose depedente ao fluconazol. Dois isolados de corrente sanguínea foram resistentes, um ao fluconazol e o outro à micafungina. Todos os isolados foram capazes de produzir pelo menos um dos fatores de virulência investigados. A análise molecular por RAPD-PCR revelou cepas idênticas que estavam nas mãos de profissionais distintos, e também revelou um cluster com cinco cepas altamente similares (*Sj* > 80%) de *C. parapsilosis stricto sensu*, sendo quatro do ambiente e uma de corrente sanguínea. **Conclusões:** Espécies de *Candida* foram isoladas de mãos e ambiente mesmo após higienização e desinfecção. O ambiente pode ter sidofonte de infecção, já que isolados de ambiente e infecção apresentaram perfis genéticos similares. Alguns isolados de mãos apresentaram alta similaridade genética e estavam em diferentes profissionais de saúde e grupos, destacando a importância da higiene das mãos paraminimizar o risco de contaminação cruzada em UTIN, e evidenciando a importância de medidas básicas mais rigorosas para controle de infecção hospitalar com o objetivo de prevenir a transmissão nosocomial.

Palavras-chave: mãos, *Candida*, ICS, fatores de virulência, biofilme.

ABSTRACT

Introduction: Bloodstream infection caused by *Candida* spp. it is recognized as one of the main causes of morbidity and mortality in newborns. Studies carried out around the world show an association between infections acquired in the hospital environment and the practice of washing hands and inanimate reservoirs. This fact causes the environmental transmission of virulent microorganisms and the concern for the safety of the newborn becomes even greater. Therefore, epidemiological assessment of infections in the NICU is necessary. **Objective**: to analyze *Candida* species isolated from the hands of healthcare professionals, before and after cleaning with 70% ethyl alcohol-based gel, from the environment and from bloodstream infections in the NICU, and evaluate virulence factors, biofilm production and genetic similarity. **Methodology**: Isolates were identified using MALDI-TOF. Antifungal susceptibility tests were performed in accordance with documents from the Clinical and Laboratory Standard Institute. The isolates were studied to produce extracellular hydrolytic enzymes (hemolysin, Dnase, proteinase and phospholipase) through phenotypic tests (egg yolk agar, bovine albumin agar, 7% sheep blood agar and Dnase agar); biofilm formation and metabolic activity were assessed using crystal violet and XTT staining, respectively. Genetic similarity was performed using randomly amplified polymorphic DNA (RAPD-PCR) methodology. Epidemiological surveillance was carried out through the "National Healthcare Safety Network" system. **Results**: *C. parapsilosis complex* was the most common species. Three isolates obtained from the hands of professionals were resistant to amphotericin B, one was resistant to micafungin and five were resistant to fluconazole. One environmental isolate showed dose-dependent susceptibility to fluconazole. Two bloodstream isolates were resistant, one to fluconazole and the other to micafungin. All isolates were capable of producing at least one of the investigated virulence factors. A molecular analysis by RAPD-PCR revealed identical strains that were in the hands of different professionals, and also revealed a cluster with five highly similar strains (Sj > 80%) of C. *parapsilosis stricto sensu*, four from the environment and one from the bloodstream. **Conclusions**: *Candida* species were isolated from hands and the environment even after hygiene and disinfection. The environment may have been a source of infection, as isolates from the environment and infections contain similar genetics. Some hand isolates had high genetic similarity and were in different healthcare professionals and groups, highlighting the importance of hand hygiene to minimize the risk of cross-contamination in NICUs, and highlighting the importance of more rigorous basic measures to control hospital-acquired infections. The objective of preventing nosocomial transmission.

Keywords: hands, *Candida*, ICS, virulence factors, biofilm.

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LISTA DE ABREVIATURAS E SIGLAS

AAM	Alta Atividade Metabólica
ABHSs	Antissépticos para as mãos à base de álcool
APB	Alta Produção de Biomassa
ASD	Agar Sabouraud Dextrose
BAM	Baixa Atividade Metabólica
BHI	Infusão de Cérebro e Coração
BPB	Baixa Produção de Biomassa
BrSCOPE	Vigilância e controle Brasileiro de patógenos de importância epidemiológica
CEP	Comitê de Ética em Pesquisa com Seres Humanos
CIM	Concentração Inibitória Mínima
CLSI	Clinical Laboratory Standards Institute
CNCA	*Candida* não-*Candida albicans*
CV	Cristal Violeta
CVC	Cateter Venoso Central
CVP	Cateter Venoso Periférico
DO	Densidade Ótica
DNA	Ácido Desoxirribonucleico
DNase	deoxyribonuclease
EBP	Extremo Baixo Peso
HC-UFU	Hospital de Clínicas da Universidade Federal de Uberlândia
ICS	Infecção de Corrente Sanguínea
IFI	Infecções Fúngicas Invasivas
IRAS	Infecções Relacionadas à Assistência à Saúde
MALDI-TOF	Tempo de voo de dessorção/ionização a laser assistida por matriz
MAM	Moderada Atividade Metabólica
MBP	Muito Baixo Peso
MIC	Concentração Inibitória Mínima
MPB	Moderada Produção de Biomassa
NPT	Nutrição Parenteral Total
NHSN	National Healthcare Safety Network

PBS	Solução salina tamponada com fosfato
PCR	Reação em Cadeia pela Polimerase
PICC	Cateter Central de Inserção Periférica
PS	Profissionais de Saúde
R	Resistente
RAPD	DNA polimórfico amplificado aleatoriamente
RPMI	Roswell Park Memorial Institute
S	Sensível
SDD	Susceptibilidade Dose Dependente
UFC	Unidades Formadoras de Colônia
UFU	Universidade Federal de Uberlândia
UTIN	Unidade de Terapia Intensiva Neonatal
XTT	(2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5Carboxanilide)
YPD	Yeast, Peptona e Dextrose

SUMÁRIO

1. INTRODUÇÃO

As micoses invasivas são responsáveis todos os anos por mais de dois milhões de infecções em todo o mundo e por, pelo menos, tantas mortes quanto à tuberculose ou a malária. A tuberculose causa cerca de 1,5 milhões de mortes por ano, enquanto a malária resulta em aproximadamente 600 mil mortes anualmente. Candidíase, aspergilose, criptococose e pneumocistose causam mais de 90% das mortes relatadas associadas a micoses invasivas. (QUINDOS, 2018; ATIENCIA-CARRERA et al., 2022).

Dentre elas, a micose mais frequente é a candidemia ou infecção de corrente sanguínea (ICS) por Candida, que é principalmente uma condição associada aos avanços médicos na área de terapia intensiva. É reconhecida como uma das principais causas de morbidade e mortalidade em recém-nascidos (RN) com muito baixo peso ao nascer (MBP) e extremo baixo peso ao nascer. O muito baixo peso ao nascer (MBP) refere-se a bebês que nascem com menos de 1.500 gramas, enquanto o extremo baixo peso ao nascer se refere a bebês que nascem com menos de 1.000 gramas. Esses bebês são particularmente vulneráveis a infecções devido ao seu sistema imunológico imaturo e à necessidade frequente de procedimentos médicos invasivos (EBP) (QUINDOS, 2018; ATIENCIA-CARRERA et al., 2022; DA SILVA, 2023).

A mortalidade neonatal mundial é estimada em 31 por 1000 nascidos vivos. Nos países em desenvolvimento a frequência de ICSem RN prematuros varia de 30% a 60%, sendo a taxa demortalidade entre 40% e 70%. No Brasil há cerca de três milhões de nascimentos a cada ano, com aproximadamente 7 a 10% desses RN necessitando de cuidados intensivos ou especiais durante algum tempo. A Unidade de Terapia Intensiva Neonatal (UTIN) oferece suportes vitais que possibilitam a sobrevivência de muitos recém-nascidos (RN) prematuros. Esses suportes incluem ventilação mecânica para ajudar na respiração, incubadoras para manter a temperatura corporal, nutrição parenteral para fornecer nutrientes essenciais, monitoramento contínuo de sinais vitais, e administração de medicamentos e fluidos intravenosos. Cerca de 25% dos prematuros e de baixo peso desenvolvem ao menos um episódio de sepse até o 3° dia de vida, sendo a pele a principal porta de entrada. (ARAÚJO et al., 2012; PAULA; SALGE; PALOS, 2017).

Após 48 horas do nascimento, os tecidos do recém-nascido (RN) são invadidos por micro-organismos de origem predominantemente materna. Subsequentemente, micro-organismos originários da equipe de saúde, de outras crianças e de visitantes podem se instalar, e o RN pode desenvolver infecções exógenas ou mesmo infecções adquiridas. As

infecções exógenas são aquelas contraídas a partir de fontes externas, como o ambiente hospitalar ou contato com outras pessoas, enquanto infecções adquiridas são aquelas contraídas após o nascimento devido a fatores externos ou internos, como procedimentos médicos invasivos. (ARAÚJO et al., 2012).

As ICS são as infecções neonatais mais frequentes na UTIN (45-55%), seguidas deinfecções respiratórias (16-30%) e infecções do trato urinário (8-18%). A baixa idade gestacional e o peso ao nascer são os riscos individuais mais identificados (SCHWAB et al., 2007; COUTO et al., 2007). Dados de epidemiologia regional mostraram uma incidência de 2,49 por 1.000 admissões no Brasil (BARANTSEVICH e BARANTSEVICH, 2022), e uma taxa de sobrevida de 27,8% em um estudo brasileiro (SANTOLAYA et al., 2019).

Candida spp. são responsáveis por 80% de todas as infecções fúngicas sistêmicas e são a quarta, sexta e sétima principais causas de ICS nos EUA, Europa e Brasil, respectivamente (CANELA et al., 2018). A espécie mais comum ainda é a *Candida albicans*, mas a incidência de espécies de *Candida* não-albicans (CNCA) tem aumentado (DADAR et al., 2018). A candidemia por *C. parapsilosis* e *C. tropicalis* é mais frequente que a candidemia por *C. albicans* em alguns hospitais, especialmente em instituições de países em desenvolvimento e em hospitais com unidades de terapia intensiva neonatal. Esses hospitais muitas vezes enfrentam desafios adicionais, como recursos limitados, infraestrutura hospitalar sobrecarregada e maior uso de dispositivos médicos invasivos, o que pode contribuir para o aumento da incidência de infecções por CNCA.

Algumas espécies, como *C. glabrata* e *C. krusei*, são menos suscetíveis a alguns antifúngicos e esse aumento está relacionado ao uso generalizado de antifúngicos. A prevalência de espécies também tem sido associada à região geográfica, setor hospitalar e perfil do paciente, ressaltando a necessidade de estudos locais (DADAR et al., 2018).

Aproximadamente, 50% das infecções microbianas são associadas com formações de biofilme, que aumentam significantemente a resistência aos antimicrobianos e aos mecanismos de defesa do sistema imune (ATIENCIA-CARRERA et al., 2022). A resistência do biofilme nas leveduras é explicada por uma matriz de pequenas colônias de leveduras e pseudohifas dispostas em uma estrutura bilateral, que promove maior resistência aos antifúngicos (NUCCI; COLOMBO, 2002; KAUFMAN et al., 2014; ATIENCIA-CARRERA et al., 2022), essa resistência nas UTIN podem ter consequências desastrosas para os RN afetados, já que a terapia empírica não é mais efetiva (WYNN et al., 2012).

Estudos realizados em todo o mundo têm mostrado a associação das infecções adquiridas no ambiente hospitalar à prática inadequada de higienização das mãos e à

reservatórios inanimados (CAMPANARO et al., 2016; SORIA et al., 2016; VAN ASBECK et al., 2007; HERNÁNDEZ-CASTRO et al., 2010). Estima-se que 1,7 milhões de infecções estejam associadas ao cuidado em saúde e, deste, 100.000 mortes associadas a infecções (CAMPANARO et al., 2016).

Esse fato ocasiona a veiculação ambiental de micro-organismos virulentos e a preocupação com a segurança dos RN torna-se ainda maior. As evidências sinalizam para falhas envolvendo a higienização das superfícies ambientais, no processo de trabalho, aliados à falta de uma política organizacional focada na segurança do paciente e do trabalhador. Essas falhas contribuem para a cadeia epidemiológica ambiental, contaminação cruzada e colonização dos RN e trabalhadores dos hospitais em detrimento da falta de qualidade na assistência (SILVA et al., 2016).

Nesse sentido, os trabalhadores de saúde, na condição de colonizados por micro-organismos em decorrência das atividades no ambiente de trabalho e muitos deles resistentes aos antimicrobianos, passam a veicular esses agentes para pacientes e outros trabalhadores. Tornando assim, participantes da cadeia epidemiológica das infecções relacionadas à assistência à saúde (IRAS) (SILVA et al., 2016).

A tipagem molecular é uma técnica eficiente usada para examinar a estrutura populacional e as características epidemiológicas de patógenos fúngicos, fornecendo informações valiosas sobre a dinâmica das infecções por *Candida* spp. (DADAR et al., 2018). A avaliação do parentesco genético tornou-se muito mais fácil com a disseminação dos métodos de tipagem molecular, podendo auxiliar também na identificação da fonte (ambiental ou pessoal) de infecção (CAMPIONI et al., 2015). Embora uma variedade de estratégias baseadas em PCR (polymerase chain reaction) tenha sido desenvolvidas para fins de impressão digital de DNA, a técnica RAPD-PCR (Random Amplified Polymorphic DNA) é um dos métodos mais populares para impressão digital de DNA de espécies fúngicas de interesse médico (SOLL, 2000; CAMPIONI et al., 2015). Muitas das espécies que são principais causas de infecção adquiridas no hospital também são organismos comensais e, portanto, é importante determinar se o isolado recuperado do paciente é uma cepapatogênica que causou a infecção ou uma contaminante comensal que provavelmente não é a fonte da infecção (SINGH et al., 2006; TSAI, 2015).

A avaliação epidemiológica das infecções em UTIN se faz necessário, em relação ao tratamento profilático, principalmente em recém-nascidos em situação de risco, prematuros e de baixo peso, sendo de questão central na gestão de infecção hospitalar (LOPÉZ et al., 2013; VISWANATHAN et al., 2012; PAMMI et al., 2014). Frente a esta situação se faz necessário

medidas de prevenção e controle, como higiene das mãos entre os profissionais de saúde, manutenção da técnica asséptica nos procedimentos, detecção precoce de portadores de infecção, e busca ativa de RN colonizados (CARVALHO et al., 2014).

2. FUNDAMENTAÇÃO TEÓRICA

2.1 Recém-nascido

O recém-nascido (RN), mesmo em ótimas condições, nasce susceptível às infecções devido ao seu sistema imunológico ser naturalmente imaturo. Ótimas condições referem-se a um ambiente de parto seguro e higiênico, onde a mãe teve uma gravidez saudável e acesso a cuidados médicos adequados, e onde o RN nasce a termo, com bom peso e sem complicações de saúde imediatas. Desde o momento do parto, o RN inicia seu processo de colonização por agentes virulentos e continua esse processo pelo contato com a mãe, familiares, a equipe multiprofissional, além do contato com objetos utilizados na assistência, muitas vezes contaminados, como termômetros, estetoscópios, incubadoras, e outros transdutores. (SCHWAB et al., 2007; COUTO et al., 2007).

Aproximadamente 50% dos lactentes internados em UTIN podem estar colonizados no final da primeira semana de vida, eisso aumenta para 64% nas quatro próximas semanas de vida do RN (LEGEAY, 2015; ATIENCIA-CARRERA et al., 2022). Os recém-nascidos prematuros, particularmente não possuem barreira efetiva da pele, possuem um sistema imune imaturo, requerendo apoio e hospitalização prolongados.

Os avanços nos cuidados pré-natais e neonatais têm contribuído significativamente para a melhoria das taxas de sobrevivência de recém-nascidos em situações críticas, muitas vezes com o suporte de tecnologias e equipamentos sofisticados. A ventilação mecânica, por exemplo, desempenha um papel crucial no tratamento de recém-nascidos com dificuldades respiratórias, oferecendo suporte vital por meio de diferentes modos e técnicas adaptadas às necessidades individuais dos bebês. (PEREIRA; CUNHA, 2013; KINSEY et al., 2017; ARAÚJO et al., 2012).

O suporte nutricional também é uma área fundamental, com a nutrição parenteral e enteral sendo utilizadas para fornecer os nutrientes necessários ao crescimento e desenvolvimento dos recém-nascidos. Da mesma forma, o suporte cardiovascular, incluindo o uso de monitores de pressão arterial e sistemas como a ECMO (Oxigenação por Membrana Extracorpórea), é crucial para manter a função cardíaca e a circulação em bebês com instabilidade cardiovascular. Esses avanços têm permitido que muitos bebês que antes não teriam chances de sobrevivência possam ser tratados e se recuperem com sucesso (PEREIRA; CUNHA, 2013; KINSEY et al., 2017;ARAÚJO et al., 2012).

No entanto, o uso prolongado de suporte invasivo pode estar associado a uma série de complicações e efeitos adversos a longo prazo. Por exemplo, a ventilação mecânica pode causar danos pulmonares crônicos e problemas respiratórios persistentes, enquanto a presença de dispositivos invasivos aumenta o risco de infecções hospitalares. Essas complicações podem impactar a qualidade de vida dos recém-nascidos e representar desafios adicionais para os cuidados a longo prazo (PEREIRA; CUNHA, 2013; KINSEY et al., 2017; ARAÚJO et al., 2012).

O avanço da medicina neonatal continua a ser impulsionado pela inovação tecnológica e pela abordagem multidisciplinar, que envolve profissionais de saúde colaborando para fornecer um cuidado abrangente e coordenado. Embora o suporte invasivo seja essencial para salvar vidas, é crucial que as decisões sobre seu uso sejam feitas com base em considerações éticas e no cuidado centrado na família, garantindo que os pais estejam envolvidos nas decisões e recebam o suporte necessário durante o tratamento de seus filhos. (PEREIRA; CUNHA, 2013; KINSEY et al., 2017;ARAÚJO et al., 2012).

Considerando o ambiente da UTIN, os principais fatores de risco associados ao desenvolvimento de candidemia em RN, são prematuridade, baixo peso ao nascer, condições imunossupressoras (incluindo imaturidade da pele e do trato gastrointestinal), permanência hospitalar prolongada, nutrição parenteral total (NPT), uso de dispositivos de terapia intensiva (ventilação mecânica, cateter venoso central (CVC)) e exposição a medicamentos que promovem o crescimento de fungos (corticosteroides pós-natais e antibióticos de amplo espectro) (SILVA et al. ,2023).

A incidência de ICS por *Candida* spp. em RN com EBP ao nascer varia de 2-20%, com mortalidade variando de 25-40%. O peso ao nascer e a idade gestacional são os fatores de risco não modificáveis que predizem a mortalidade em ICS por *Candida* spp. (ANUREKHA, 2018). O estudo de Kelly et al. (2015) descreveu que 40% de mortalidade é observada em pacientes com menos de 750 gramas de peso ao nascer e 20% de mortalidade em RN de 1.000 a 1.500 gramas de peso ao nascer. Neonatos com ICS por *Candida* spp. apresentam mortalidade três vezes maiorque os recém-nascidos não infectados de idade gestacional e peso semelhantes (ANUREKHA,2018).

2.2 Infecção de Corrente Sanguínea (candidemia)

A candidemia é caracterizada como a presença de espécies de *Candida* na corrente sanguínea. A colonização prévia da mucosa e da pele é o primeiro passo para o desenvolvimento

da infecção. Colonização neonatal por *Candida* spp. é secundária à transmissão materna ou aquisição nosocomial pelas mãos dos profissionais de saúde na UTIN. *Candida albicans* é o patógeno fúngico mais prevalente (LEGEAY, 2015).

Vários fatores de risco estão associados à candidemia: MBP ao nascer; uso de linhas centrais; intubação; NP; administração de antibióticos de amplo espectro; hospitalização prolongada; cirurgia abdominal; e colonização prévia (DOI et al., 2016; LEGEAY, 2015). A candidemia é uma importante causa de morbidade e mortalidade em UTIN. Portanto, para reduzir a ocorrência e melhorar o prognóstico, é extremamente necessário detecção precoce e manejo prudente da candidemia em RN (DA SILVA, 2023).

A epidemiologia das candidemias em Unidades de Terapia Intensiva Neonatal (UTIN) é fundamental para entender a incidência, os fatores de risco e as estratégias de prevenção dessa infecção fúngica em recém-nascidos. A candidemia, causada por fungos do gênero *Candida*, é uma das principais causas de sepsis fúngica em recém-nascidos, especialmente em UTIN. A incidência global varia entre um e 10 casos por 1.000 recém-nascidos admitidos, sendo particularmente alta em bebês prematuros. No Brasil, a taxa estimada é de cerca de 2 a 4 casos por 1.000 recém-nascidos, o que destaca a relevância do problema (LEGEAY, 2015).

Os principais fatores de risco para candidemia incluem a prematuridade, o uso extensivo de antibióticos, a presença de procedimentos invasivos como cateteres intravenosos e condições clínicas graves. Bebês prematuros, devido à imaturidade do sistema imunológico e à necessidade de intervenções frequentes, estão especialmente vulneráveis. A antibioticoterapia, embora essencial para tratar infecções bacterianas, pode alterar a flora microbiana e favorecer o crescimento de *Candida*. Além disso, o uso de dispositivos invasivos e a presença de condições subjacentes aumentam o risco de infecção (DA SILVA, 2023).

O diagnóstico de candidemia em recém-nascidos pode ser desafiador devido à apresentação clínica variável e à necessidade de testes laboratoriais específicos. A cultura de sangue é o método padrão para confirmar a infecção, mas técnicas diagnósticas mais rápidas, como a detecção de antígenos ou DNA de *Candida*, estão em desenvolvimento para melhorar a rapidez e a precisão do diagnóstico. O tratamento geralmente envolve antifúngicos, com o fluconazol sendo uma escolha comum. Em casos graves, antifúngicos mais potentes, como anfotericina B ou equinocandinas, podem ser necessários (LEGEAY, 2015).

Para prevenir a candidemia, são essenciais estratégias como a higiene rigorosa, o cuidado adequado com cateteres intravenosos, o uso prudente de antibióticos e o

monitoramento vigilante. A adesão a práticas de higiene e a manutenção correta dos dispositivos invasivos ajudam a reduzir a incidência de infecções. Globalmente, a candidemia neonatal apresenta variações na incidência e nas práticas de manejo, com países desenvolvidos frequentemente exibindo taxas mais baixas devido a melhores recursos e práticas. A compreensão aprofundada da epidemiologia das candidemias é crucial para formular políticas de saúde eficazes e melhorar o manejo dessas infecções em recém-nascidos (LEGEAY, 2015).

2.3 Espécies de *Candida*

Cada espécie de *Candida* apresenta características diferentes, como morfologia, composição da parede celular, assimilação de nutrientes, produção de atributos de virulência e suscetibilidade a antifúngicos (CANELA et al., 2018; DADAR et al., 2018). Por isso, é importante identificar com precisão as espécies, devido as suas peculiaridades.

2.3.1 Candida albicans

C. albicans é um membro da microbiota humana, e caracteriza-se como uma levedura polimórfica diplóide de superfícies mucosas, sendocomumente encontrada nos tratos gastrointestinal, respiratório e geniturinário humano. Geralmente é um fungo comensal inofensivo que pode setornar um organismo oportunista em indivíduos imunocomprometidos ou imunologicamente deficientes. A transição de levedura para hifa determina estados comensais e patogênicos de *C. albicans* e pode levar à infecção de tecidos, evasão de macrófagos, adesão de células hospedeiras e desenvolvimento de comunidades de biofilme. Foi demonstrado que infecções sistêmicas por *C. albicans* levam a uma taxa de mortalidade de aproximadamente 40%. A morbidade associada às infecções por *C. albicans* depende principalmente das respostas imunes associadas do hospedeiro e dos tecidos/órgãos afetados (DADAR et al., 2018). *C. albicans* parece ser mais virulento do que outras espécies de *Candida* e freqüentemente surge de fontes endógenas (translocação do trato gastrointestinal e genital), que são menos afetadas pelas medidas de controle da infecção (DADAR et al., 2018). Os fatores de risco mais importantes para infecções por *C. albicans* são antibioticoterapia seguida de acesso venoso central, procedimentos cirúrgicos, neutropenia, nutrição parenteral, cateter urinário, bem como algumas doenças como malignidade hematológica, câncer sólido,

prematuridade, doença cardíaca, trauma, doença neurológica, doença gastrointestinal, transplante de órgão, doença pulmonar, doença vascular, HIV, doença genética/malformação congênita, doença renal, diabetes mellitus, doença hepática e doença pancreática. Em indivíduos gravemente imunocomprometidos, *C. albicans* induz infecção sistêmica e pode passar de infecções locais oportunistas ou comensais da boca, garganta e tratoreprodutivo para uma candidíase invasiva sistêmica afetando o sistema circulatório, ossos e cérebro (DADAR, 2018). Um estudo de coorte de 12 anos na China (HSU et al., 2018), revelou que 47,8% das infecções invasivas em RN foram por *C. albicans*. Outro estudo de Piqueras et al. (2020) de oito anos na Espanha, revelou que a candidíase invasiva ocorreu principalmente em lactentes da UTIN (36,0%), nos quais *C. albicans* foi a espécies mais frequente (oito de 12 casos - 53,0%), e os lactentes EBP foram os mais afetados.

2.3.2 Candida parapsilosis

C. parapsilosis é o patógeno comensal humano mais comum, é particularmente adquirida de fontes exógenas, persiste em ambientes hospitalares, é transmitido por mãos contaminadas e a colonização prévia não é um pré-requisito para causar infecção invasiva (PIQUERAS, 2020; MARIA, 2018). *C. parapsilosis* foi reconhecida desde 2005, como um complexo representado por três espécies, *C. parapsilosis sensu stricto*, *C. metapsilosis* e *C. orthopsilosis*. Como as três espécies são filogeneticamente e morfologicamente indistinguíveis, só é possível diferenciá-las com métodos proteômicos e/ou moleculares (DA SILVA et al., 2023; MARIA et al., 2018).

Mudanças recentes na epidemiologia da candidemia foram documentadas, incluindo uma proporção crescente de candidíase neonatal por CNCA, especialmente pelo complexo *C. parapsilosis*. Na pesquisa de DA SILVA et al., (2023), as espécies desse complexo foram o agente etiológico de candidemia mais isolado na UTIN, representando 38,6% dos casos, seguido por *C. albicans* (31,8%). O principal reservatório de *C. parapsilosis* no ambiente da UTIN permanece desconhecido, mas a transmissão tem sido associada às mãos dos profissionais de saúde ou a cateteres ou soluções de nutrição parenteral contaminados. Além disso, está levedura tem a capacidade de produzir biofilme na superfície de dispositivos intravasculares, como os CVC (DA SILVA et al. 2023; RIERA et al., 2022).

C. parapsilosis é conhecida por sua resistência relativa a alguns antifúngicos, o que complica o tratamento e o manejo das infecções. A resistência a azóis, como o fluconazol, e a capacidade de formar biofilmes são características que contribuem para a dificuldade de

erradicação da infecção. Esses fatores destacam a importância de estratégias de controle e prevenção rigorosas, além da necessidade de monitoramento contínuo para garantir a eficácia dos tratamentos antifúngicos e das práticas de controle de infecção (MARIA et al., 2018).

2.3.3 Candida guilliermondii

C. guilliermondii é um grupo geneticamente heterogêneo de espécies de levedura fenotipicamente indistinguíveis, incluindo *C. guilliermondii*, *C. fermentati*, *C. carpophila* e *C. xestobii*. A identificação precisa e oportuna dos isolados do complexo *C. guilliermondii* em nível de espécie, incluindo os perfis de suscetibilidade aos antifúngicos associados, é essencial para orientar as decisões clínicas (CHENG, 2016; HIRAYAMA et al., 2018).

A incidência de candidemia por *C. guilliermondii* varia de 1% a 3%, dependendo da região geográfica (CHENG, 2016; TSENG et al., 2018). No entanto, os poucos relatos disponíveis sobre infecções por *C. guilliermondii* indicam que esses organismos estão associados a resultados clínicos ruins, refletindo a gravidade e o impacto dessas infecções, particularmente em pacientes críticos e imunocomprometidos (CHENG, 2016). A alta mortalidade é uma das principais preocupações, com taxas de mortalidade frequentemente elevadas devido à virulência dessa espécie de *Candida* (CHENG, 2016).

Outra questão crítica é a resistência a antifúngicos. *Candida guilliermondii* pode demonstrar resistência a alguns antifúngicos, como os azóis, tornando o tratamento mais difícil e limitando as opções terapêuticas disponíveis. Essa resistência pode levar a uma resposta inadequada ao tratamento padrão, exigindo abordagens terapêuticas mais agressivas e prolongadas, o que aumenta o risco de complicações e prolonga o tempo de hospitalização (CHENG, 2016).

C. guilliermondii faz parte da microbiota da pele e mucosas humanas, e raramente é reconhecida como um patógeno invasivo (TSENG et al., 2018). Apesar de poucos estudos relatarem ICS por essa espécie, nas duas últimas décadas houve um crescimento significativo, o estudo de TSENG, et al., (2018) em Twaian, relatou 36 pacientes com fungemia e a idade média dos pacientes foi de 50,5 anos (variando de 17 dias a 96 anos). Em resumo, embora *Candida guilliermondii* seja uma causa menos comum de candidemia em UTIN, seu impacto clínico significativo e a resistência a antifúngicos tornam a gestão dessa infecção um desafio importante. A compreensão da epidemiologia local e global ajuda a formular estratégias eficazes para a prevenção e tratamento dessas infecções em recém-nascidos.

2.3.4 *Candida glabrata*

C. glabrata é considerada uma espécie comensal comum nos tratos gastrointestinal geniturinário, mas pode tornar-se um patógeno fúngico oportunista em pacientes imunocomprometidos (SAVASTANO, 2016). *C. glabrata* apresenta um potencial de multirresistência, demonstrando uma frequência crescente de resistência a equinocandinas e azóis (BARANTSEVICH; BARANTSEVICH, 2022). Além disso, pode apresentar resistência inata e adquirida frente a drogas antifúngicas (tais como, caspofungina e fluconazol), devido à sua capacidade de modificar a biossíntese de ergosterol, função mitocondrial ou efluxo antifúngico (CANELA et al., 2018).

No estudo de Pandita et al. (2017) na Índia, *C.glabrata* foi a espécie mais isolada nos neonatos, e o baixo peso e prematuridade foram os fatores de risco mais associados. ICS causada por esta espécie está associada a altas taxas de mortalidade. No estudo de Canela et al. (2018), em um hospital terciário no Brasil, a taxa de mortalidade bruta em pacientes adultos foi de 73%, enquanto na Índia (GUPTA; VARMA.; GUPTA, 2015), a taxa de mortalidade hospitalar em 30 dias foi de 53,8%.

Colombo et al. (2013) em um estudo multicêntrico no Brasil mostrou que *C. glabrata* está emergindo como um patógeno relevante no Brasil, responsável por 10% de todos os episódios. O mesmo resultado foi mostrado por Doi et al. (2016) no Brasil, onde *C. glabrata* foi responsável por 10% de todos os episódios de candidemia.

2.3.5 *Candida tropicalis*

C. tropicalis foi descrita como primeira causa comum de candidemia na Índia, e segunda causa comum no Brasil, são países onde a grande maioria dos casos são tratados com fluconazol, devido ao alto custo das equinocandinas (ARASTEHFAR et al., 2020; DALAL; BABU; ANURADHA, 2021). No entanto, um número crescente de estudos de candidemia mostrou um aumento significativo de isolados sanguíneos de *C. tropicalis* resistentes a azóis.

Embora *Candida tropicalis* não seja tão predominante quanto em alguns países em desenvolvimento, os Estados Unidos e países europeus têm observado resistência crescente entre isolados de *C. tropicalis*. Nesses locais, a resistência a azóis pode variar, mas o problema é menos pronunciado em comparação com regiões onde o uso de fluconazol é mais frequente. A resistência relatada nos Estados Unidos e na Europa tende a estar mais

relacionada ao uso de antifúngicos em ambientes hospitalares e à seleção de cepas resistentes em pacientes imunocomprometidos (ANURADHA, 2021).

O aumento da resistência a azóis entre isolados de *Candida tropicalis* é um problema significativo que afeta o tratamento e o manejo de candidemia em várias regiões do mundo. A necessidade de alternativas terapêuticas e de estratégias de controle de infecção mais rigorosas é evidente para enfrentar esse desafio crescente (ANURADHA, 2021).

Os pacientes infectados com *C. tropicalis* apresentam hospitalização mais longa e maior mortalidade em comparação com aqueles infectados com *C. albicans* (MEGRI et al. 2020; ARASTEHFAR et al., 2020). A capacidade desse organismo de produzir aglomerados é um de seus principais fatores de virulência. Uma vez introduzida no hospedeiro imunocomprometido,
C. tropicalis pode ser mais virulenta do que *C. albicans* e pode progredir rapidamente da colonização para a invasão (ARASTEHFAR et al., 2020).

C. tropicalis como causa de fungemia em UTIN tem sido associada à presença do fungo nas mãos dos profissionais em hospitais (ARASTEHFAR et al., 2020). Por outro lado, outros sugerem que é adquirido de origem ambiental fora do ambiente hospitalar (MEGRI et al. 2020).

Candida tropicalis é uma das principais causas de candidemia globalmente, com uma prevalência notável em países em desenvolvimento. Na Índia, essa espécie é a principal responsável por infecções fúngicas invasivas, enquanto no Brasil ocupa a segunda posição após *Candida albicans*. A taxa de incidência de candidemia por *Candida tropicalis* pode variar entre 10% e 30% dos casos, refletindo sua importância clínica. A infecção é particularmente prevalente em Unidades de Terapia Intensiva Neonatal (UTIN), onde fatores como prematuridade e o uso de dispositivos invasivos aumentam a vulnerabilidade dos recém-nascidos (ARASTEHFAR et al., 2020).

A mortalidade associada a infecções por *Candida tropicalis* é significativa, com taxas que podem alcançar 20% a 47%, dependendo da gravidade da infecção e da resistência aos antifúngicos. A resistência crescente a azóis, como o fluconazol, tem exacerbado o problema, dificultando o tratamento e contribuindo para desfechos clínicos piores. Pacientes com cepas resistentes enfrentam uma maior probabilidade de complicações graves e um aumento na mortalidade, destacando a necessidade urgente de estratégias eficazes de controle e tratamento (MEGRI et al. 2020; CANELA et al., 2018).

2.4 Espécies raras/atípicas

Na última década, leveduras emergentes e atípicas foram relatadas como uma causa aumentada de infecções fúngicas em pacientes imunocomprometidos e/ou hospitalizados. (KUMAR et al., 2022).

C. famata é uma levedura comensal encontrada em queijos, laticínios e no meio ambiente. É uma causa rara de candidíase, respondendo por apenas 0,2%–2% dos isolados coletados de estudos de vigilância antifúngica. Apresenta CIM elevadas a antifúngicos sendo uma preocupação no tratamento de candidíase invasiva devido a essa espécie (BEYDA et al., 2013; KUMAR et al., 2022).

C. norvegensis foi identificada a primeira vez em 1954, no escarro de três pacientes asmáticos, na Noruega (DIETRICHSON, 1954). Desde então, apenas alguns casos de infecção por *C. norvegensis* foram relatados, ocorrendo principalmente em pacientes com câncer ou HIV (KUMAR et al., 2022).

A candidíase por *C. haemulonii* está associada à doença vascular periférica, diabetes mellitus e úlceras crônicas nas pernas, e a resistência à anfotericina B, itraconazol e fluconazol. Equinocandinas, posaconazol e voriconazol demonstraram potente atividade *in vitro* contra esta espécie (COLES et al., 2020).

C. pararugosa é um patógeno de levedura emergente e raro relatado em humanos e animais em diferentes órgãos e líquidos biológicos. *C. pararugosa* parece levar principalmente à ICS, predominantemente em crianças e adultos (KUMAR, et al. 2022). A candidemia causada por *C. pararugosa* está associada à alta morbidade e mortalidade, especialmente entre pacientes imunocomprometidos (KUMAR et al., 2022; EL HELOU; PALAVECINO, 2017).

C. pelliculosa é um patógeno fúngico raro encontrado principalmente no solo, lagos, frutas fermentadas e poluentes industriais. *C. pelliculosa* é um patógeno oportunista que causa infecções em hospedeiros imunocomprometidos, particularmente RN prematuros EBP ao nascer e hospitalizados na UTIN, resultando em surtos de candidemia neonatal (CAI; WEI; CHENG, 2021; KUMAR et al., 2022).

C. catenulata é uma levedura ascomicetosa, contaminante de produtos lácteos e tem sido associada a uma ingestão alimentar e invasiva em humanos e animais. C. catenulata geralmente não está associada a doenças em humanos. No entanto, casos raros foram descritos em paciente com câncer, e em infecções vulvovaginais (CHEN, 2021; O'BRIEN et al., 2018, RHIMI et al. 2021).

2.5 Fatores de Virulência

A patogenicidade nas espécies de *Candida* é atribuída a um conjunto de fatores de virulência, incluindo a capacidade de aderir a células endoteliais, transição de levedura para hifa (morfogênese, fenômeno típico de *C. albicans*), a produção de enzimas (proteinases, fosfolipases e hemolisinas, e Dnase), expressão de adesinas e invasões na superfície celular, a mudança fenotípica, a formação de biofilme e a capacidade de evadir células do sistema imunológico (VIEIRA DE MELO et al., 2019; DADAR et al., 2018).

2.5.1 Biofilme

As infecções nosocomiais estão intimamente associadas ao crescimento de biofilmes aderidos a dispositivos médicos ou tecidos do hospedeiro. Os biofilmes são o estado de crescimento predominante de muitos microrganismos, sendo uma comunidade de células aderentes irreversíveis com propriedades fenotípicas e estruturais diferentes quando comparadas às células flutuantes (planctônicas). Os Institutos Nacionais de Saúde estimam que os biofilmes sejam responsáveis, de uma forma ou de outra, por mais de 80% de todas as infecções microbianas nos Estados Unidos (ATIENCIA-CARRERA et al., 2022).

As espécies de *Candida* podem produzir biofilmes bem estruturados compostos por vários tipos de células e até mesmo espécies microbianas, levando a uma resistência intrínseca contra uma ampla variedade de fatores de estresse, como vários antifúngicos e mecanismos de defesa imunológica. (ATIENCIA-CARRERA et al., 2022).

A formação de biofilmes em dispositivos médicos tem várias implicações clínicas. Primeiramente, infecções associadas a biofilmes são mais difíceis de tratar, exigindo frequentemente a remoção do dispositivo infectado além do tratamento antifúngico. Isso pode resultar em complicações graves e prolongar o tempo de hospitalização. A presença de biofilmes também está associada a uma maior taxa de infecção e a um aumento na morbidade e mortalidade dos pacientes. Em ambientes de UTIN, onde a utilização de dispositivos invasivos é comum, o problema é ainda mais crítico, pois neonatos com sistemas imunológicos imaturos estão particularmente vulneráveis a infecções associadas a biofilmes (ATIENCIA-CARRERA et al., 2022).

No estudo de Atiencia-Carrera e colaboradores (2022), cerca de 70,0% das notificações de candidemia foram causadas por estirpes formadoras de biofilme, e a taxa de mortalidade associada às cepas formadoras de biofilme foi de 70,0% nas infecções da corrente sanguínea relacionadas à *Candida*. Essas infecções são difíceis de tratar porque as células fúngicas dentro do biofilme são cobertas por uma matriz extracelular que reduz fisicamente a concentração do

fármaco antifúngico no biofilme e controla o crescimento por meio de mecanismos de quorum sensing. Por outro lado, como a formação de biofilme é um dos sistemas pelos quais os fungos sobrevivem no ambiente, sabe-se que é causa de infecções nosocomiais se o manejo ambiental adequado não for realizado (MIYAKE et al., 2022).

Para mitigar os problemas associados a biofilmes, é crucial adotar estratégias eficazes de controle e prevenção. Isso inclui a manutenção rigorosa de práticas de higiene e desinfecção para minimizar a contaminação dos dispositivos médicos. A utilização de dispositivos impregnados com substâncias antimicrobianas pode ajudar a reduzir a formação de biofilmes. Além disso, a educação contínua dos profissionais de saúde sobre a importância da inserção e manejo adequado dos dispositivos é essencial para reduzir o risco de infecções associadas a biofilmes (ATIENCIA-CARRERA et al., 2022).

Em resumo, a formação de biofilmes em dispositivos médicos representa um desafio significativo no ambiente hospitalar, aumentando a resistência a tratamentos e complicando o manejo de infecções. A compreensão e a abordagem proativa desse problema são essenciais para melhorar a segurança dos pacientes e a eficácia dos tratamentos em ambientes críticos.

2.5.1 Enzimas hidrolíticas extracelulares

As enzimas hidrolíticas extracelulares desempenham um papel importante no crescimento de *Candida*, uma vez que facilitam a adesão e penetração tecidual e, consequentemente, a invasão no hospedeiro, potencializando sua patogenicidade (ERUM et al., 2020; CANELA et al., 2018; RIERA et al., 2022; DABIRI et al. 2018). Essas enzimas tambémestão envolvidas na ligação de espécies de *Candida* ao tecido-alvo, especialmente no estágio de hifas (FATHI et al., 2022).

A epidemiologia das infecções associadas a biofilmes em ambientes hospitalares revela que essas infecções são um problema significativo, especialmente em contextos com uso extensivo de dispositivos médicos invasivos. Espécies de *Candida*, como *Candida albicans*, *Candida glabrata* e *Candida tropicalis*, são frequentemente responsáveis pela formação de biofilmes em dispositivos médicos e são conhecidas por produzir enzimas que contribuem para a sua patogenicidade (DABIRI et al. 2018).

Candida albicans é uma das espécies mais comuns associadas à formação de biofilmes. Ela possui a capacidade de produzir uma série de enzimas, como as aspartil proteases e fosfolipases, que desempenham um papel crucial na adesão e na formação do biofilme. Essas enzimas ajudam a quebrar as barreiras físicas e químicas, facilitando a adesão

da levedura às superfícies dos dispositivos e promovendo a formação de biofilmes robustos. *Candida glabrata* também é um agente significativo, conhecido por sua habilidade em formar biofilmes e por apresentar uma maior resistência a tratamentos antifúngicos, o que torna as infecções mais difíceis de erradicar. *Candida tropicalis*, apesar de ser menos comum, também contribui para infecções associadas a biofilmes e está associada a uma alta taxa de resistência a antifúngicos em algumas regiões (DABIRI et al. 2018).

Em termos de epidemiologia, as infecções associadas a biofilmes estão em ascensão devido ao uso crescente de dispositivos médicos invasivos em ambientes hospitalares. As taxas de infecção podem variar, mas o problema é exacerbado por fatores como a resistência a antifúngicos e a falta de opções de tratamento eficazes para biofilmes estabelecidos. A prevalência dessas infecções reflete a complexidade da gestão de infecções fúngicas em ambientes críticos, onde a presença de biofilmes e a produção de enzimas pelos patógenos desempenham papéis centrais na patogênese e na dificuldade de tratamento (DABIRI et al. 2018).

As fosfolipases extracelulares atuam na membrana da célula hospedeira, o que resulta na ruptura ou na modificação dos atributos de superfície que promovem a adesão e penetração das membranas celulares do hospedeiro e consequente infecção. As proteinases têm capacidade de hidrolisar proteínas do hospedeiro, como albumina, imunoglobulina e proteínas da pele (ERUM et al., 2020; RIEIRA et al., 2022). A fosfolipase e a proteinase são consideradas os dois fatores de virulência mais comuns, pois contribuem para a interação *Candida*-hospedeiro (PANDEY; GUPTA; TILAK, 2018).

A hemolisina degrada os glóbulos vermelhos, liberando ferro que é absorvido pelas células da levedura. Esta propriedade de virulência capaz de adquirir ferro degradando a hemoglobina do hospedeiro propicia ao patógeno uma maior capacidade de persistir e sobreviver (PANDEY; GUPTA; TILAK, 2018).

A atividade da Dnase (deoxyribonuclease) extracelular como fator de virulência para fungos ainda é indefinido. Segundo Riceto et al. (2015) as hipóteses são de que a Dnase contribua para a evasão do sistema imunológico, evitando a morte da levedura que pode ser causada por neutrófilos, e que a Dnase pode ser importante em combinação com outras enzimas para degradar o DNA de outros micro-organismos no microambiente, especialmente bactérias, facilitando a local de colonização pela redução da competição microbiana.

2.6 Resistência antifúngica

Além da determinação dos fatores de risco para candidemia em RN e da correta taxonomia do patógeno, é fundamental identificar resistência antifúngica em espécies de *Candida*, a fim de auxiliar na escolha do tratamento mais adequado (DA SILVA et al., 2023).

Infecções por *Candida* spp. tornaram-se mais difíceis de tratar na última década devido ao uso desproporcional de drogas imunossupressoras, desnutrição, distúrbios endócrinos, uso generalizado de dispositivos médicos de longa permanência, antibióticos de amplo espectro (ATIENCIA-CARRERA et al., 2022; BARANTSEVICH; BARANTSEVICH, 2022).

Os azóis (fluconazol, itraconazol, voriconazol, posaconazol, ravuconazol e isavuconazol) geralmente demonstram atividade fungistática contra *Candida* spp. inibindo a lanosterol 14 demetilase, uma enzima chave da biossíntese do ergosterol. A resistência ao fluconazol é mais comum em *C. auris*, *C. glabrata* e *C. parapsilosis*; *C. krusei* tem resistência intrínseca ao fluconazol. A resistência a outros azóis raramente é encontrada (BARANTSEVICH; BARANTSEVICH, 2022).

As equinocandinas (micafungina, anidulafungina, caspofungina) representam a mais nova classe de antifúngicos e apresentam efeito fungicida em espécies de *Candida.* Seu mecanismo de ação é a inibição da-D-glucana sintase: enzima importante na síntese da parede celular. Todas as equinocandinas têm alta eficiência em infecções invasivas e um alto perfil de segurança (BARANTSEVICH; BARANTSEVICH, 2022; DA SILVA et al., 2023).

A anfotericina B é um polieno que se liga ao esgosterol o principal componente da membrana celular fúngica, criando poros e subsequente morte celular, e tem sido usado com sucesso no tratamento de diferentes infecções fúngicas invasivas, incluindo candidemia. A maioria das espécies de *Candida* são susceptíveis à anfotericina B. No entanto, a resistência é frequentemente detectada em *C. lusitaniae*. A anfotericina B e suas formulações lipídicas são os únicos polienos sistêmicos disponíveis com formulações lipídicas menos tóxicas e mais bem toleradas pelos pacientes. E o uso convencional é limitado pela intolerância individual e nefrotoxicidade frequentemente encontradas. (BARANTSEVICH; BARANTSEVICH, 2022)

2.7 Infecções relacionadas à assistência à saúde (mãos e ambiente)

As IRAS ocorrem em todo o mundo e afetam tanto países desenvolvidos quanto países com recursos escassos. As infecções adquiridas em ambientes de assistência à saúde estão entre as principais causas de morte e aumento de estados de morbidez em doentes hospitalizados. Elas representam uma carga significativa tanto para os doentes e suas famílias quanto para a

saúde pública. A falha na aplicação de medidas de controle de infecções favorece a disseminação de patógenos. Esta disseminação pode ser especialmente importante durante surtos, e os ambientes de assistência à saúde podem servir como multiplicadores de doenças, causando impacto tanto nos hospitais quanto na saúde da comunidade (RAI et al., 2019; KRATZEL et al., 2020; FALLICA et al., 2021).

A prevenção de infecções associadas a biofilmes em ambientes hospitalares é essencial para garantir a segurança dos pacientes e a eficácia dos tratamentos. A higienização das mãos é uma das medidas mais importantes nesse contexto. A lavagem frequente das mãos com água e sabão ou a utilização de álcool gel reduz significativamente a carga microbiana, impedindo a transferência de patógenos para dispositivos médicos e superfícies do hospital. A prática correta e regular da higienização das mãos é fundamental para prevenir a disseminação de infecções, especialmente em ambientes críticos onde o uso de dispositivos invasivos é comum (KERI et al. 2021).

O álcool gel, com uma concentração de 60-70% de álcool, é uma solução eficaz para a desinfecção das mãos, especialmente quando a lavagem com água e sabão não está disponível. Ele é capaz de eliminar uma ampla gama de microrganismos, incluindo bactérias e fungos, e deve ser utilizado antes e após o contato com pacientes e ao manusear dispositivos médicos. No entanto, é importante lembrar que o álcool gel pode não ser eficaz contra todos os patógenos e não substitui a lavagem das mãos quando estas estão visivelmente sujas (KERI et al. 2021).

Além da higienização das mãos, a manutenção e cuidados adequados com os dispositivos médicos são cruciais para a prevenção de biofilmes. Isso inclui a utilização de dispositivos com agentes antimicrobianos, a limpeza e desinfecção regular dos mesmos e a remoção de dispositivos desnecessários assim que possível. Um programa eficaz de controle de infecções, que inclua vigilância ativa e protocolos de resposta, ajuda a monitorar e controlar surtos de infecções associadas a biofilmes. Em suma, a adesão rigorosa às práticas de higienização e ao controle de infecções é vital para minimizar o risco de infecções e proteger a saúde dos pacientes em ambientes hospitalares (KERI et al. 2021).

As mãos dos profissionais de saúde são o veículo mais importante para a transmissão de patógenos (REF) A contaminação das mãos pode ocorrer durante o contato direto com o pacienteou por meio do contato indireto com produtos e equipamentos no ambiente próximo a este, como bombas de infusão, barras protetoras das camas e estetoscópio, entre outros (BOYCE; PITTET, 2002; KERI et al. 2021).

A higienização das mãos é uma medida básica para reduzir as infecções. Embora a

ação seja simples, a baixa adesão entre os profissionais da saúde é um problema em todo o mundo (FALLICA et al., 2021; BOYCE; PITTET, 2002; WORLD HEALTH ORGANIZATION; WHO PATIENT SAFETY, 2009). O estudo de Keri et al. (2021) com vários profissionais de saúde de enfermaria e UTI demonstraram que 33,3% dos profissionais que representava um terço dos profissionais de saúde amostrados, apresentaram carga fúngica nas mãos.

A higienização das mãos, de acordo com as principais organizações de saúde, deve ser realizada em várias situações críticas para garantir a segurança e prevenir infecções. É essencial higienizar as mãos antes e depois de qualquer contato direto com pacientes, bem como após a remoção de luvas. Deve-se também proceder com a lavagem das mãos antes de manusear instrumentos invasivos e após o contato com fluidos corporais, excrementos, membranas ou mucosas, pele não intacta ou curativos. Além disso, é importante higienizar as mãos ao mudar de uma área contaminada para uma área limpa do corpo durante o cuidado com o paciente, bem como após o contato com objetos nas proximidades imediatas do paciente. Essas práticas são fundamentais para manter um ambiente seguro e reduzir o risco de transmissão de infecções (KRATZEL et al., 2020; FALLICA, 2021; BOYCE; PITTET, 2002; WORLD HEALTH ORGANIZATION; WHO PATIENT SAFETY, 2009).

A higiene das mãos é fundamental na prevenção de infecções nosocomiais, mas a descolonização eficaz e a desinfecção das superfícies ambientais das UTIN também são necessárias para reduzir a transmissão, controlar e gerenciar surtos associados à assistência à saúde, uma vez que as superfícies ambientais da UTIN abrigam grande número de bactérias e fungos associados a IRAS em neonatos (ELKADY et al., 2022).

As IRAS também estão associadas ao ambiente hospitalar e equipamentosmédicos. Embora a higiene das mãos seja importante para minimizar o impacto dessa transferência, limpar e desinfetar superfícies ambientais, é fundamental para reduzir sua contribuição potencial para o incidência de IRAS (ELKADY et al., 2022). A triagem ambientalconfirma a contaminação repetida de itens, equipamentos e locais gerais, em leitos e quartos depacientes colonizados ou infectados e, muitas vezes, em várias áreas clínicas em uma instituiçãode saúde (15). As mãos dos profissionais de saúde podem tocar essas superfícies contaminadasdurante o atendimento ao paciente, o que aumenta o risco de transmissão para outras pessoas (DANCER, 2014).

3 OBJETIVOS

Os objetivos deste estudo foram:

- Verificar a presença de isolados de *Candida* spp. nas mãos dos profissionais de saúde da UTIN antes e após a higienização com gel à base de álcool e isolados do ambiente;
- Investigar as infecções invasivas na cultura de sangue dos neonatos e os resultados davigilância epidemiológica (NHSN);
- Determinar a sensibilidade dos isolados de leveduras frente aos antifúngicos;
- Caracterizar fenotipicamente os isolados de leveduras através da avaliação da morfologia e da pesquisa de produção das enzimas hidrolíticas extracelulares (hemolisina, DNAse, proteinase efosfolipase);
- Pesquisar a formação de biofilme, e atividade metabólica;
- Analisar o perfil de similaridade molecular dos isolados de levedura através da metodologia de RAPD-PCR.

Artigo 1 – "Virulence factors, antifungal susceptibility and molecular profile in Candida species isolated from the hands of health professionals before and after cleaning with 70% ethyl alcohol-based gel"

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Research Paper

Virulence factors, antifungal susceptibility and molecular profile in *candida* species isolated from the hands of health professionals before and after cleaning with 70% ethyl alcohol-based gel

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ABSTRACT

Fungal infections in neonatal intensive care units (NICU) are mainly related to *Candida* species, with high mortality rates. They are predominantly of endogenous origin, however, cross-infection transmitted by healthcare professionals' hands has occurred. The aim of this study was to identify *Candida* species isolated from the hands of healthcare professionals in a NICU before and after hygiene with 70% ethanol-based gel and evaluate virulence factors DNase, phospholipase, proteinase, hemolysin, biofilm biomass production, and metabolic activity. In vitro antifungal susceptibility testing and similarity by random amplified polymorphic DNA (RAPD) were also performed. *C. parapsilosis* complex was the most frequent species (57.1%); all isolates presented at least one virulence factor; three isolates (*Candida parapsilosis* complex) were resistant to amphotericin B, two (*Candida famata* [currently *Debaryomyces hansenii*] and *Candida guilliermondii* [currently *Meyerozyma guilliermondii*]) was resistant to micafungin, and six (*Candida parapsilosis* complex, *Candida guilliermondii* [=*Meyerozyma guilliermondii*], *Candida viswanathii, Candida catenulata* [currently *Diutina catenulata*] and *Candida lusitaniae* [currently *Clavispora lusitaniae*]) were resistant to fluconazole. Molecular analysis by RAPD revealed two clusters of identical strains that were in the hands of distinct professionals. *Candida* spp. were isolated even after hygiene with 70% ethanol-based gel, highlighting the importance of stricter basic measures for hospital infection control to prevent nosocomial transmission.

Introduction

Fungal infections that occur in Neonatal Intensive Care Units (NICU) are mainly related to *Candida* species, which is the seventh most prevalent cause (5.6%) of bloodstream nosocomial infections (BSI) among all pathogens included in the Brazilian Surveillance and Control of Pathogens of Epidemiologic Importance (BrSCOPE) [1,2]. Invasive *Candida* infections can be fatal, especially in hospitalized newborns in NICU, due to an immature immune system influenced by healthcare, such as central venous catheters, broad-spectrum antibiotics, and parenteral nutrition [3].

The hospital environment is a large reservoir of opportunistic pathogens, which can be transmitted to individuals in many ways [4]. The modes of transmission and portals of entry for hospital fungal infections vary according to the pathogen involved [4]. *Candida* spp. infections are predominantly of endogenous origin [4], but cross-infection transmitted by healthcare professionals' hands (HCWs) has

Abbreviations: NICU, neonatal intensive care units; RAPD, random amplified polymorphic DNA; BrSCOPE, brazilian surveillance and control of pathogens of epidemiologic importance; HCW, healthcare professionals; ABHSs, alcohol-based hand antiseptics; LBP, low biomass production; MBP, moderate biomass production; HBP, high biomass production; LMA, low metabolic activity; MMA, moderate metabolic activity; HMA, high metabolic activity

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been reported in 20–40% of healthcare-associated infections (HAIs) [4,5] and is considered one of the main sources of candidemia outbreaks in NICU [6,7]. The ability of *Candida* isolates to adhere and grow as biofilms in internal medical devices and intravascular catheters, for example, has been associated with higher mortality [5,8–10], since from these reservoirs, persistent *Candida* cells (tolerant to antifungals) tend to migrate to the bloodstream [11–14].

Hand hygiene and antiseptic practices for *Candida* infection control, according to the main world health organizations, are handwashing with soap and water, especially with dirt, followed by using alcohol-based hand antiseptics (ABHSs) [10–13,15–17]. In this context, the aim of this study was to evaluate the presence of *Candida* spp. before and after hand hygiene of healthcare professionals in a NICU with 70% ethanol-based gel, as well as to phenotypically characterize the isolates and determine their genetic similarity.

Materials and methods

Study site, participant selection, and sample collection

This prospective cohort study was carried out in the NICU of a tertiary teaching hospital in Brazil with 514 beds and a multidisciplinary team of 120 healthcare professionals, including nurses, nursing technicians, doctors, and physiotherapists. Of these, 107 (89.1%) professionals participated in the study. Hand samples were collected from HCWs before and after disinfection with 70% ethyl alcohol-based gel (Hydrated 70% v/v Ethanol Gel, Rioquímica®, Brazil), which is used in the unit. HCWs were instructed to immerse both hands in a sterile polypropylene bag containing 30 mL of Brain Heart Infusion (BHI) broth (HIMEDIA®, India) and rub their hands in the broth. The bag was sealed and labeled as the "before" group. Afterward, the professionals dried their hands with a sterile surgical compress and disinfected their hands with alcohol gel according to the institution's protocol. They then introduced both hands into another sterile polypropylene bag containing 30 mL of BHI broth and rubbed their hands in the broth. The bag was sealed and labeled as the "after" group [17].

Isolation and identification of species

The entire sample obtained was transferred to sterile Falcon tubes and centrifuged at, 4000 g for 10 min. Subsequently, the supernatant was discarded, and the sediment was resuspended in 0.9% saline at a 1:1 ratio. 0.1 mL was inoculated in plates containing Sabouraud Dextrose Agar (SDA; Isofar, Duque de Caxias, RJ, Brazil) supplemented with chloramphenicol and chromogenic agar (Himedia, Mumbai, India) and incubated at 30 °C for up to 72 h [18]. The SDA plates were used for enumerating colony-forming units per milliliter (CFU/mL). The count was performed on plates that showed growth from a single colony to evaluate the efficacy of 70% ethanol-based gel.

The yeast species were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker MALDI Biotyper 4.0).

Evaluation of in vitro virulence factors

Extracellular enzymatic activity

The production of phospholipase, proteinase, hemolysin, and DNase was determined according to the methods described by Rorig et al. [19], with adaptations. Briefly, yeast colonies were suspended in 0.9% saline solution with turbidity equivalent to tube two of the McFarland scale (3×10^8 to 6×10^8 CFU/mL). Subsequently, five μL aliquots of each suspension were deposited at equidistant points on Petri dishes (90 × 15 mm) containing egg yolk agar for phospholipase, bovine albumin agar for proteinase, 7% sheep blood agar for hemolysin, and DNase agar (Hexis, São Paulo, Brazil). Incubation was performed at 30 °C for four days for phospholipase, seven days for proteinase and DNase, and 48 h for hemolysin. Tests were performed in duplicate and on two different occasions. The interpretation was carried out according to Menezes et al. [20].

Biofilm formation

Biofilm production was detected according to Pierce et al. [21] and Costa-Orlandi et al. [22], with modifications. Briefly, 10 μL of a 24-hour culture in SDA from each isolate was inoculated into 20 mL of Yeast Extract Peptone-Dextrose (YPD) broth and incubated overnight at 35 ± 2 °C. After incubation, the tubes were centrifuged at 5000 rpm for five minutes, the supernatant was removed, and the pellet was resuspended in 20 mL of phosphate-buffered saline (PBS), a process that was repeated three times. Then, 1 mL of 0.9% saline was added to the remaining pellet, 20 μL of this suspension was pipetted into a Neubauer chamber, and the cell density was adjusted to 1.0×10^6 cells/mL. After counting, 10–100 μL of the inoculum was added to 900–1000 μL of Roswell Park Memorial Institute (RPMI) 1640 broth (Himedia, Mumbai, India) supplemented with glucose and MOPS buffer (Hexis, São Paulo, Brazil) and deposited 100 μL into flat-bottom 96-well plates and incubated for 24 h at 35 ± 2 °C.

For biofilm quantification, two plates were incubated, one for biomass production quantification and the other for metabolic activity evaluation, following Pierce et al. [21] and Costa-Orlandi et al. [22]. After 24 h incubation, the supernatant was gently removed, and the wells of both plates were washed three times with PBS to remove planktonic cells. Biofilm biomass production quantification was performed by adding 100 μL of 0.5% Crystal Violet and incubating at room temperature for five minutes. Then, the wells were washed with sterile distilled water to remove the excess dye, and 100 μL of 33% acetic acid was added after washing. The reading was done in a plate reader (Epoch-Biotek) at a wavelength of 570 nm.

For metabolic activity evaluation, 50 μL of the 2.3-bis (2-methoxy-4-nitro-5- sulfophenyl)–5- [carbonyl (phenylamino)]–2H- 151 tetrazolium hydroxide)-XTT (1 mg ml−1 of PBS) and menadione (1 mM in ethanol) solution were added to the wells and incubated for 3 h. After incubation, the reading was done in a plate reader (Epoch-Biotek) at a wavelength of 490 nm. Results were classified according to the Optical Density (OD) of each sample [23]: Crystal Violet (staining low biomass production (LBP) < 0.44; moderate biomass production (MBP) = 0.44–1.17; high biomass production (HBP) >1.17). XTT reduction (low metabolic activity (LMA) < 0.097; moderate metabolic activity (MMA) = 0.097–0.2; high metabolic activity (HMA) > 0.2) [23]. Wells containing only RPMI were the negative control. *Candida albicans* ATCC 90,028 was used as the test control. Tests were done in quadruplicate and repeated three times independently.

Antifungal susceptibility testing

The susceptibility test to fluconazole (Fluoxol, La Paz, Bolivia), amphotericin B (Cristalia, São Paulo, Brazil), and micafungin (Raffo, Buenos Aires, Argentina) was performed by the broth microdilution method as described in document M27-S4 [24]. *Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 90028 were used as the test control. The assay was performed in duplicate, and the reading was done at 490 nm.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the antifungal capable of promoting 50% reduction in yeast growth for fluconazole and micafungin and 90% for amphotericin B [24]. The MIC50 represents the value of the MIC at which at least 50% of the strains were inhibited. The MIC50 was considered the value at position n x 0.5 when n was an even number of strains. For an odd n, the value at position $(n + 1)$ x 0.5 represented the MIC50 value. The MIC90 represents the value of the MIC at which at least 90% of the strains were inhibited. The MIC90 was calculated using n x 0.9. When the result was an integer, that number

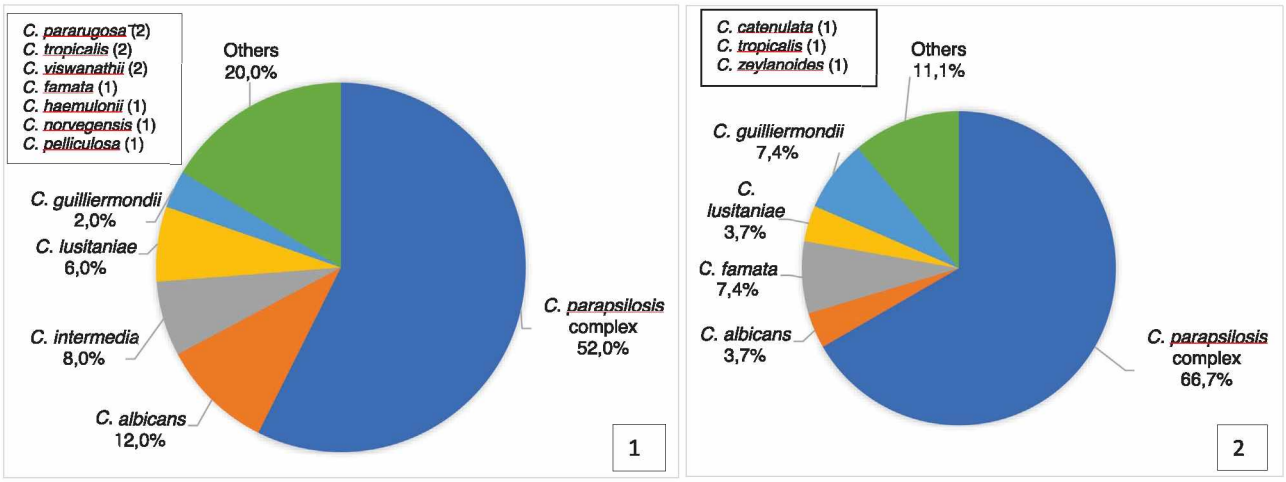

Fig. 1. *Candida* species isolated from the hands of healthcare professionals 1 - before and 2 - after cleaning with 70% ethyl alcohol-based gel.

represented the MIC90; for a result where the number was not an integer, the next integer after the respective value represented the MIC90 [25]. MIC50 and MIC90 were calculated only for *C. parapsilosis* isolates, as they had an $n > 10$. The interpretation of MIC and the cut-offs for each antifungal were considered according to the CLSI M27-S4, M60, and M59 documents [24,26,27]. Due to the lack of clinical breakpoint (CBPs) or epidemiological cutoff values (ECVs) by CLSI for amphotericin B, micafungin, and fluconazole regarding *C. catenulata* (= *Diutina catenulata*), *C. intermedia, C. pararugosa* (currently *Wickerhamiella pararugosa*), *C. lusitaniae* (=*Clavispora lusitaniae*), *C. norvegensis* (currently *Pichia norvegensis*), *C. famata* (= *Debaryomyces hansenii*), *C. pelliculosa* (currently *Wickerhamomyces anomalus*), *C. haemulonii* (currently *Candida haemuli*), *C. viswanathii*, and *C. zeylanoides* species, isolates with MIC for amphotericin $B \leq 2$ μg/mL were considered low MIC and MIC > 2 μg/mL high MIC; isolates with MIC for fluconazole ≤ 8 μg/mL were considered low MIC and MIC > 8 μg/mL was considered high MIC, and MIC for micafungin ≤1.0 μg/mL were considered low MIC and those with MIC of ≥2 μg/mL were considered high MIC [28].

RAPD (random amplified polymorphic DNA)

Genomic DNA extraction was performed for all isolates obtained from SDA media (24 h) at 35 °C according to Bolano et al. [29]. For RAPD-PCR analysis, the primers OPA9 (5′-GGGTAACGCC-3′), OPA18 (5′-AGCTGACCGT-3′), OPB11 (5′-GTAGACCCGT-3′), and OPG17 (5′-ACGACCGACA-3′) (Operon Technologies Inc.) were used. Reactions and amplification products were carried out according to the protocol of Riceto et al. [30]. The gels were observed under ultraviolet light (L-Pix HE, Loccus Brasil, Cotia, SP, Brazil) and images were captured using the photodocumentation system (Image Lab-1D Loccus Brasil, Cotia, SP, Brazil). Bands were represented as present (1) or absent (0) and analyzed using the Multivariate Statistical Package (MVSP) version 3.21 statistical program (Kovach Computing Services, UK.). Genetic relationships were calculated using the Jaccard coefficient (Sj). Sj values of 1.00 and 0.99 represent the same genotype, values between 0.80 and 0.99 represent highly similar but not identical samples and values below 0.80 represent distinct samples [30]. Dendrograms based on Sj values were generated by the UPGMA method (Unweighted Pair-Group Method with Arithmetical Averages).

Statistical analysis

Quantitative variables were described within the group before and after hand hygiene with 70% ethyl alcohol-based gel, using mean, median, maximum, and minimum standard deviation. Additionally, the Shapiro-Wilk normality test was applied. For variables that showed normal distribution in both groups, the t-Student test was used to compare them; for those that did not show normal distribution, the Mann-Whitney test was applied [31].

Qualitative variables were described (frequency and percentage) through two-way tables. The associations of qualitative variables were evaluated using the likelihood ratio test/G-test [32]. P values < 0.05 were considered significant. All analyses were performed using SPSS software for Windows (version 20.0; IBM Corp., Armonk, NY, USA).

Results

Of the 107 HCWs who participated in the study, *Candida* species were isolated from the hands of 46 (43.0%) in the before a group and 24 (22.4%) in the after group. Regarding CFU, the before group ranged from $0.6 - 60.8 \times 10^4$ CFU/mL, and the after group ranged from $0.0 - 30.0 \times 10^4$ CFU/mL, with a 94.1% reduction after the use of 70% ethyl alcohol-based gel and a $p < 0.001$.

Seventy-seven *Candida* isolates from 14 distinct species were analyzed, with 50 (64.9%) from the before a group and 27 (35.1%) from the after group (Fig 1). In the before a group, seven rare/atypical species were isolated (14.0% - *C. haemulonii* [=*C. haemuli*], *C. intermedia, C. lusitaniae* [=*Clavispora lusitaniae*], *C. norvegensis* [=*Pichia norvegensis*], *C. viswanathii, C. pararugosa* [=*Wickerhamiella pararugosa*] and *C. pelliculosa* [=*Wickerhamomyces anomalus*], and three (11.1% - *C. catenulata* [= *Diutina catenulata*], *C. lusitaniae* [=*Clavispora lusitaniae*], and *C. zeylanoides*) were isolated in the after group.

Fig. 1 *Candida* species isolated from the hands of healthcare professionals before and after cleaning with 70% ethyl alcohol-based gel.

All isolates from both groups were able to form biofilms, in addition to producing at least one exoenzyme (Table 1). Of the 50 isolates from the before a group, 49 (98.0%) had HMA and 41 (82.0%) had HBP, nine (18.0%) had MBP, and one (2.0%) had MMA. Fifteen (30.0%) were positive for DNase, eight (16.0%) had phospholipase activity (five moderate and three high), six (12.0%) had proteinase activity (three high and three moderate), and two (4.0%) had hemolytic activity (moderate).

All 27 isolates from the after the group had HBP, 20 (74.1%) had HMA, five (18.5%) had MMA, and two (7.4%) had LMA. Twelve (44.4%) were positive for DNase, four (14.8%) had proteinase activity (high), two (7.4%) had hemolytic activity (moderate), and two (7.40%) had phospholipase activity (moderate).

When the two groups were compared, there was no statistically significant difference in virulence factors (Table 2).

Table 1
Phenotypic and genotypic characteristics of *Candida* spp. isolated from the hands of healthcare professionals in a neonatal intensive care unit, before and after hand hygiene with 70% ethyl alcohol-based gel.

Sample	Hemolytic activity	DNAse	Proteinase	Phospholipase	Biofilm		Antifungal susceptibility profile			Genotypic profile
					CV	XTT	Ampho B	Fluco	Mica	
Before (50)										
C. pa 2	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	M*
C. pa 4	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	M
C. pa 10	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	M
C. pa 13	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	M*
C. pa 15	Neg	Neg	Neg	High	HBP	HMA	S	S	S	M
C. pa 16	Neg	Neg	Neg	Neg	HBP	MMA	R	S	S	M
C. pa 30	Neg	Pos	Neg	Neg	HBP	HMA	R	S	S	–
C. pa 38	Neg	Neg	Neg	Neg	HBP	HMA	S	SDD	S	–
C. pa 39	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	F
C. pa 40	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	F
C. pa 52	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	L
C. pa 62	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	–
C. pa 64	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	L
C. pa 69	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	L
C. pa 74	Neg	Neg	Neg	Neg	MBP	HMA	S	S	S	–
C. pa 75	Neg	Neg	Neg	Neg	HBP	HMA	S	SDD	S	–
C. pa 88	Neg	Pos	Neg	Neg	HBP	HMA	S	SDD	S	–
C. pa 92	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	M
C. pa 98	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	K
C. pa 100	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	M
C. pa 101	Neg	Pos	Neg	Neg	MBP	HMA	S	S	S	–
C. pa 102	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	–
C. pa 111	Neg	Pos	Neg	Neg	MBP	HMA	S	R	S	–
C. pa 119	Neg	Pos	High	Neg	MBP	HMA	S	S	S	L
C. pa 139	Mod	Neg	Neg	Neg	HBP	HMA	S	S	S	J
C. pa 149	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	J
C. al 31	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	B
C. al 91	Mod	Neg	High	Neg	HBP	HMA	S	SDD	S	B
C. al 95	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	B
C. al 96	Neg	Neg	Neg	High	HBP	HMA	S	S	S	–
C. al 115	Neg	Neg	Neg	High	HBP	HMA	S	S	S	B*
C. al 143	Neg	Neg	Neg	High	HBP	HMA	S	S	S	B*
C. in 8	Neg	Neg	Neg	Neg	HBP	HMA	L	L	L	A
C. in 43	Neg	Pos	Mod	Mod	HBP	HMA	L	L	L	–
C. in 48	Neg	Neg	Mod	Neg	MBP	HMA	L	L	L	A
C. in 97	Neg	Pos	Neg	Neg	HBP	HMA	L	L	L	A
C. lu 37	Neg	Neg	Neg	Neg	HBP	HMA	L	L	L	E
C. lu 83	Neg	Pos	Neg	Neg	HBP	HMA	L	L	L	–
C. lu 140	Neg	Neg	Neg	Neg	HBP	HMA	L	L	L	E
C. gui 133	Neg	Neg	Neg	Neg	HBP	HMA	S	R	S	C
C. para 12	Neg	Neg	Neg	Neg	HBP	HMA	L	L	L	D
C. para 49	Neg	Neg	Neg	Neg	HBP	HMA	L	L	L	D
C. tro 44	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	–
C. tro 53	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	–
C. vis 82	Neg	Neg	Neg	Neg	MBP	HMA	L	H	L	–
C. vis 109	Neg	Pos	Neg	Neg	MBP	HMA	L	L	L	–
C. nor 26	Neg	Neg	Neg	Neg	HBP	HMA	L	L	L	–
C. fa 27	Neg	Neg	Neg	Neg	MBP	HMA	S	S	S	–
C. pe 50	Neg	Neg	Neg	Neg	HBP	HMA	L	L	L	–
C. hae 77	Neg	Neg	Mod	Mod	MBP	HMA	L	L	L	–
After (27)										
C. pa 3	Neg	Neg	Neg	Neg	HBP	LMA	S	S	S	–
C. pa 11	Neg	Neg	High	Neg	HBP	MMA	S	S	S	I
C. pa 21	Neg	Pos	High	Neg	HBP	HMA	R	SDD	I	H
C. pa 23	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	G
C. pa 33	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	G
C. pa 51	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	G
C. pa 55	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	–
C. pa 60	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	–
C. pa 61	Neg	Neg	Neg	Neg	HBP	LMA	S	S	S	I
C. pa 66	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	I
C. pa 73	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	I
C. pa 89	Neg	Neg	Neg	Neg	HBP	HMA	S	R	S	–
C. pa 94	Neg	Pos	Neg	Neg	HBP	HMA	S	S	S	K
C. pa 106	Neg	Neg	Neg	Neg	HBP	MMA	S	S	S	H
C. pa 108	Neg	Pos	Neg	Neg	HBP	HMA	S	SDD	S	–
C. pa 110	Neg	Neg	Neg	Neg	HBP	HMA	S	S	S	H
C. pa 118	Neg	Pos	High	Neg	HBP	HMA	S	S	S	G
C. pa 120	Neg	Neg	High	Neg	HBP	AAM	S	S	S	H
C. fa 20	Neg	Neg	Neg	Neg	HBP	AAM	S	S	S	–

(*continued*)

Table 1 (*Continued*)

Sample	Hemolytic activity	DNAse	Proteinase	Phospholipase	Biofilm		Antifungal susceptibility profile			Genotypic profile
					CV	XTT	Ampho B	Fluco	Mica	
C. fa 135	Neg	Neg	Neg	Neg	HBP	MMA	S	S	S	–
C. gui 93	Mod	Neg	Neg	Neg	HBP	MMA	S	S	S	C
C. gui 146	Mod	Neg	Neg	Neg	HBP	AAM	S	S	S	–
C. al 107	Neg	Neg	Neg	Mod	HBP	AAM	S	SDD	S	B
C. ca 46	Neg	Pos	Neg	Neg	HBP	AAM	L	H	L	–
C. lu 134	Neg	Neg	Neg	Neg	HBP	AAM	L	H	L	E
C. tro 117	Neg	Neg	Neg	Mod	HBP	AAM	L	L	L	–
C. zey 150	Neg	Neg	Neg	Neg	HBP	MMA	L	L	L	–

Note: ampho, amphotericin B; fluco, fluconazole; mica, micafungin; C.al, *Candida albicans*; C. ca, *C. catenulata (=Diutina cetnulata)*; C. pa, *C. parapsilosis*; C. para, *C. pararugosa (=Wickerhamiella pararugosa)*; C. lu, *C. lusitaniae (=Clavispora lusitaniae)*; C. in, *C. intermedia*; C. nor, *C. norvegensis (=Pichia norvegensis)*; C. fa, *C. famata (=Debaryomyces hansenii)*; C. tro, *C. tropicalis*; C. pe, *C. pelliculosa (=Wickerhamomyces anomalus)*; C. hae, *C. haemulonii (C. haemuli)*; C. vis, *C. viswanathi*; C. gui, *C. guilliermondii (=Meyerozyma guilliermondii)*; C. zey, *C. zeylanoides*; pos, positive; neg, negative; mod, moderate; XTT, tetrazolium salt; CV, crystal violet; MBP, moderate biomass production; HBP, high biomass production; LMA, low metabolic activity; MMA, moderate metabolic activity; HMA, high metabolic activity; S, susceptible; SDD susceptibility dose dependent; I, intermediate susceptibility; R, resistant; L, low MIC; H, high MIC * same genotype; - did not form genotypic profiles.

Table 2
Comparison of samples from groups before and after hand hygiene by healthcare professionals in a neonatal intensive care unit with 70% ethyl alcohol-based gel in relation to virulence factors and susceptibility profile to antifungal agents.

Classification		Before 50 (%)	After 27 (%)	Total 77 (%)	Valor p
Virulence factors					
Biomass production (CV)	MBP	1 (2.0)	0 (0.0)	1 (1.3)	2.165
	HBP	49 (98.0)	27 (100.0)	76 (98.7)	
Metabolic activity (XTT)	LMA	0 (0.0)	2 (7.4)	2 (2.3)	0.115
	MMA	9 (18.0)	5 (18.5)	14 (18.2)	
	HMA	41 (82.0)	20 (74.1)	61 (79.2)	
DNAse	positive	15 (30.0)	12 (44.4)	27 (30.7)	0.208
	negative	35 (70.0)	15 (55.6)	50 (64.9)	
Hemolytic activity	moderate	2 (4.0)	2 (7.4)	4 (5.2)	0.529
	negative	48 (96.0)	25 (92.6)	73 (94.8)	
Proteinase	high	3 (6.0)	4 (14.8)	7 (9.1)	0.134
	moderate	3 (6.0)	0 (0.0)	3 (3.9)	
	negative	44 (88.0)	23 (85.2)	67 (87.0)	
Phospholipase	high	3 (6.0)	1 (3.7)	4 (5.2)	0.511
	moderate	5 (10.0)	1 (3.7)	6 (7.8)	
	negative	42 (84.0)	25 (92.6)	67 (87.0)	
Antifungal susceptibility profile					
MIC Anfotericina B	susceptible	47 (94.0)	26 (96.3)	73 (94.8)	0.656
	resistant**	3 (6.0)	1 (3.7)	4 (5.2)	
MIC Fluconazol	susceptible	43 (86.0)	21 (77.8)	64 (83.1)	0.642
	SDD	4 (8.0)	3 (11.1)	7 (9.1)	
	resistant**	3 (6.0)	3 (11.1)	6 (7.8)	
MIC Micafungina	susceptible	49 (98.0)	26 (96.3)	75 (97.4)	0.227
	I	0 (0.0)	1 (3.7)	1 (1.3)	
	resistant**	1(2.0)	0 (0.0)	1 (1.3)	

MIC, minimum inhibitory concentration; SDD, susceptibility dose dependent; I, intermediate susceptibility; MPB, moderate biomass production; HBP, high biomass production; LMA, low metabolic activity; MMA, moderate metabolic activity; HMA, high metabolic activity. * p values < 0.05 were considered significant; **samples classified as high MIC were analyzed as resistant.

Table 1 Phenotypic and genotypic characteristics of *Candida* spp. isolated from the hands of HCWs in a NICU, before and after hand hygiene with 70% ethyl alcohol-based gel

Table 2 Comparison of samples from groups before and after hand hygiene of HCWs from a NICU with 70% ethyl alcohol-based gel in relation to virulence factors and antifungal susceptibility profile

Table 3 shows the MIC range values for the three antifungal agents tested. In the before a group, three (6.0%) isolates were resistant to amphotericin B, two (4.0%) were resistant, and one (2.0%) presented high MIC to fluconazole, and one (2.0%) isolate presented high MIC to micafungin (Tables 1 and 3). Isolates of *C. parapsilosis* complex presented an MIC50 of 0.50 μg/mL and MIC90 of 2.00 μg/mL for amphotericin B, for fluconazole they presented an MIC50 of 2.00 μg/mL and MIC90 of 4.00 μg/mL, and for micafungin they presented an MIC50 of 1.00 μg/mL and MIC90 of 2.00 μg/mL.

In the after group, one (3.7%) isolate was resistant to fluconazole and two (7.4%) presented high MIC. One (3.7%) isolate was resistant to amphotericin B (Table 1 and 3). Isolates of *C. parapsilosis* complex presented an MIC50 of 0.50 μg/mL and MIC90 of 2.00 μg/mL for amphotericin B, for fluconazole they presented an MIC50 of 1.00 μg/mL and MIC90 of 4.00 μg/mL, and for micafungin they presented an MIC50 of 1.0 μg/mL and MIC90 of 2.0 μg/mL.

There was no statistically significant difference in antifungal resistance between the two groups (Table 2).

Table 3 MIC values presented by *Candida* species on the hands of HCWs in a NICU, before and after cleaning with a 70% ethyl alcohol-based gel

Molecular analysis of the 77 isolates revealed the presence of two clusters of identical strains, represented by genotypes B* and M*, totaling four isolates, two of *C. albicans* (B* - 115 and 143) and two of

Table 3
Minimum inhibitory concentration values presented by *Candida* species in the hands of healthcare professionals in a neonatal intensive care unit, before and after cleaning with gel based on 70% ethyl alcohol.

Species (n) [currently name]	Amphotericin B MIC range (μg/mL)	Fluconazole MIC range (μg/mL)	Micafungin MIC range (μg/mL)
Before (50)			
C. parapsilosis complex (26)	0.125 – 4.00	0.50 – 8.00	0.015 – 2.00
C. pararugosa (2) [=Wickerhamiella pararugosa]	0.25 – 0.50	2.00 – 4.00	0.125 – 0.125
C. albicans (6)	0.125 – 0.50	0.50 – 4.00	0.015 – 0.03
C. famata (1) [=Debaryomyces hansenii]	1.00	1.00	2.00
C. guilliermondii (1) [=Meyerozyma guilliermondii]	0.50	8.00	1.00
C. haemulonii (1) [=C. haemuli]	4.00	4.00	0.125
C. intermedia (4)	0.06 – 0.25	1.00 – 4.00	0.03 – 0.25
C. lusitaniae (3) [=Clavispora lusitaniae]	0.50 – 1.00	0.50 – 1.00	0.25 – 0.50
C. tropicalis (2)	0.25 – 0.25	1.00 - 1.00	0.125 - 0.125
C. norvegensis (1) [=Pichia norvegensis]	1.00	1.00	0.015
C. pelliculosa (1) [=Wickerhamomyces anomalus]	0.25	0.125	0.50
C. viswanathii (2)	0.25 – 1.00	1.00 – 16.00	0.06 – 0.50
After (27)			
C. parapsilosis complex (18)	0.125 – 4.00	0.50 – 16.00	0.125 – 4.00
C. albicans (1)	0.50	4.00	0.015
C. catenulata (1) [=Diutina catenulata]	1.00	16.00	0.03
C. famata (2)	0.25 – 1.00	1.00 – 4.00	0.03 – 0.06
C. guilliermondii (2)	0.50 – 1.00	1.00 – 8.00	0.03 – 1.00
C. lusitaniae (1)	1.00	4.00	0.06
C. tropicalis (1)	0.25	0.50	0.015
C. zeylanoides (1)	0.50	0.50	1.00

MIC, minimum inhibitory concentration.

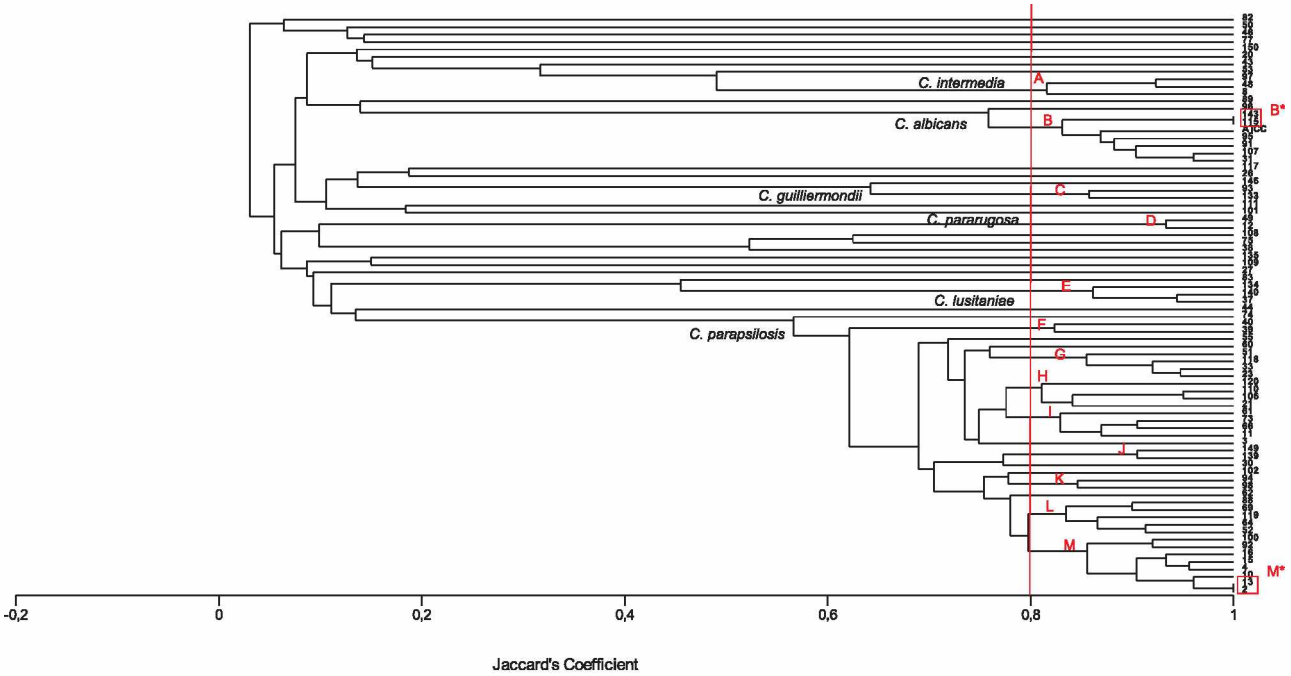

Fig. 2. RAPD dendrogram of the 77 hand samples of health professionals before and after cleaning with a 70% ethyl alcohol-based gel.

C. parapsilosis complex (M* - 2 and 13), all from the before a group and from different HCWs. The remaining isolates that showed high similarity were grouped into 13 clusters, i.e., 13 identified profiles from A to M (47/77 – 61.0%). These and other results are presented in Fig 2 and Table 1.

Cluster B is composed of six isolates of *C. albicans*, two of which (31 and 107) are highly similar and come from different groups and HCWs (Fig. 2 and Table 1).

Cluster C, formed by two *C. guilliermondii* (=*Meyerozyma guilliermondii*) isolates (93 and 133), showed high similarity, and came from different groups and professionals.

In cluster E, two *C. lusitaniae* (=*Clavispora lusitaniae*) isolates (37 and 140) found in the before a group and from different professionals, showed high similarity with *C. lusitaniae* 134, isolated from the hands of another professional in the after group (Fig. 2 and Table 1).

In cluster K, two *C. parapsilosis* complex isolates (94 and 98) were found in the hands of two HCWs and different groups (before and after, respectively). The other *C. parapsilosis* complex isolates that showed high similarity at the before moment (17/26, 65.4%) were grouped into clusters F, J, L, and M, while the remaining *C. parapsilosis* complex isolates from the after a moment (12/18, 67.0%) were grouped into clusters G, H, and I (Fig. 2 and Table 1).

Discussion

Candida spp. related HAIs are a public health problem due to their severity and high morbidity and mortality rates [33–35]. Lack of hand hygiene or inadequate hand hygiene by HCWs has been reported as one of the main reasons for the horizontal transmission of virulent *Candida* species, responsible for invasive infections in critical patients such as neonates [35,36]. Despite the importance of *Candida* species in the occurrence of HAIs, only two studies in Brazil [10,11], one in the UK [36], and two in India [8,37] have evaluated the action of alcohol-based gel in the inactivation of these species on the hands of HCWs.

This is the first study to investigate the presence of *Candida* spp. on the hands of HCWs in a NICU before and after hand hygiene with 70% ethyl alcohol-based gel. Of the 107 HCWs who participated in the study, 70 (65.4%) had contaminated hands, with 24 (34.3%) positive after hand hygiene. The 70% ethyl alcohol-based gel used in this study was able to reduce the number of CFUs, demonstrating the efficacy of its use in hand hygiene. This technique is easy to use and handle, requires less time for application, and can be used at the bedside. However, *Candida* samples were isolated from the after group, and this could have happened due to factors such as the amount of product versus friction time, which may not have been sufficient for the elimination of yeasts [15,16,34], and whether hands were wet at the time of application [15,16] or greater resistance to the antiseptic [13]. In addition, biofilm production present in all isolates in this study may have contributed to a greater permanence of yeasts in the environment, allowing them to adhere to biomedical devices and making it difficult to remove these microorganisms from hands [8,33].

In this study, *C. parapsilosis* complex and *C. albicans* were the most isolated species from the hands of HCWs, followed by rare species such as *C. guilliermondii* (=*Meyerozyma guilliermondii*), *C. famata* (=*Debaryomyces hansenii*), *C. haemulonii* (=*C. haemuli*), *C. intermedia*, and *C. lusitaniae* (=*Clavispora lusitaniae*). In the last decade, emerging and atypical yeasts have been reported as a cause of increased fungal infections in immunocompromised and/or hospitalized patients, and these pathogens in some cases have been reported with an important profile of resistance to antifungal drugs, especially azoles, and increased resistance to polyenes and echinocandins [38].

The evaluation of the virulence factors of microorganisms on the hands of HCWs is crucial, as these microorganisms can be responsible for triggering invasive infections in critical patients such as neonates. This was observed in our study published in 2018 [20], in which a case of candidemia caused by *C. albicans* in the NICU showed similarities with the other *C. albicans* isolates collected from the hands of HCWs. Other studies conducted in NICUs in Brazil [39], Mexico [40], and Portugal [41] have also identified *C. parapsilosis* species in the hands of HCWs, and these isolates were responsible for outbreaks of candidemia in neonates.

In this study, all isolates had at least one virulence factor among those analyzed. DNase, an extracellular hydrolytic enzyme that contributes to microbial pathogenicity by enhancing its adhesion, tissue damage, and evasion of the immune system, as well as its dissemination [42], was present in almost all species isolated from the hands of HCWs. *C. catenulata* (=*Diutina catenulata*) was the only species that did not produce any enzymes, but it was a strong producer of biomass, showed high metabolic activity, and was high MIC to fluconazole (MIC 16 μg/mL). The study by Chen et al. [43] showed a high MIC of this species for fluconazole (>8 μg/mL) in invasive infection samples, mainly in ICU patients. Candidemia caused by unusual species seems to have a poorer response to antifungal treatment due to MIC values above the epidemiological cutoff and a longer duration of candidemia [34].

All samples were able to form a biofilm, 87.5% with HBP and 90.9% with HMA. These data corroborate the fact that even after hand hygiene with 70% ethyl alcohol-based gel, *Candida* species were still isolated. The persistence of pathogens in biofilm in the hospital environment or in living tissues can be a source of cross-transmission with a marked clinical impact, leading to hospital-acquired infections that are more difficult to treat and have a higher chance of recurrence [44,45].

The majority (88.3%) of isolates were susceptible to the tested antifungals (amphotericin B, fluconazole, and micafungin). However, *C. parapsilosis* complex isolates showed resistance to amphotericin B and fluconazole and exhibited intermediate susceptibility to micafungin. Although resistance to echinocandins is generally low among *C. parapsilosis* complex, the increased use of this class of antifungals has resulted in changes in species prevalence and a gradual increase in minimum inhibitory concentrations of echinocandins among several species [46]. In our study, *C. haemulonii* (=*C. haemuli*) presented a high MIC (4 μg/mL) for amphotericin B. This yeast is a rare subtype, reported as an emerging and virulent pathogen, difficult to identify due to its phenotypic similarity with other *Candida* subtypes, which may increase the risk of inadequate antimicrobial administration and worsening of emerging resistance patterns [47].

Forty-six (59.8%) isolates were highly similar (Sj > 80%) in the four primers tested. Two isolates of *C. albicans* (C. al 115 and 143) and two isolates of *C. parapsilosis* complex (C. pa 2 and 13) showed genetic similarities between different HCWs in the group before hand hygiene, and these isolated, despite not producing any extracellular hydrolytic enzymes and being sensitive to the tested antifungals, presented HBP and HMA. *C. albicans* is the main pathogenic species and the most frequent cause of candidiasis in humans [48], and *C. parapsilosis* complex is a major concern because it is a frequent colonizer of human skin and hospital environments [13] and is the main *Candida* species responsible for a significant proportion of nosocomial fungemia outbreaks, particularly in NICUs [4,5,13].

C. intermedia showed similarity between two HCWs in the group before hand hygiene and had virulence factors such as moderate production of proteinase, DNase, and HBP formation. *C. intermedia* is an unusual species found in the microbiota of the human oropharyngeal cavity, and although there are no reports of infection by this species in neonates, has been isolated from the bloodstream of pediatric [49] and adults [50,51] patients with infections associated with the presence of intravenous catheters.

In this study, *C. lusitaniae* (=*Clavispora lusitaniae*) was found to have similarities between isolates of HCWs before and after hand hygiene, with the presence of virulence factors such as HBP and HMA, and the isolate after hand hygiene also showed high MIC (4 μg/mL) to fluconazole. However, *C. lusitaniae* is another rare species, only occasionally related to episodes of candidemia, corresponding to 0.2–9.4% of all *Candida* isolates in blood or other sterile sites [37,52], and unlike our results, in other studies [37,52] this species showed resistance to amphotericin B and sensitivity to fluconazole and was able to produce phospholipase, hemolysin, and proteinase enzymes.

This study has some limitations. Materials were not collected from the hands of the same HCWs in different groups, and genetic sequencing of the *Candida* isolates was not performed to confirm cross-transmission. A suggestion for future studies would be molecular analysis of isolates that showed resistance to the antifungals tested to characterize the mechanisms of resistance.

It is important to note that HCWs have a wide possibility of hand contamination during routine activities, and when not properly sanitized, increase the possibility of cross-transmission of pathogens between patients and/or professionals, with a negative impact on the entire healthcare system [15,16,53] including NICU.

In conclusion, *Candida* species were isolated from the hands of most HCWs in a tertiary hospital's NICU in Brazil in both groups (before and after), but the 70% ethyl alcohol-based gel sanitizer used in this study was effective in reducing the isolates. The most frequent species was the *C. parapsilosis* complex. All isolates produced at least

one virulence factor, with biofilm being present in all species, showing HBP and HMA. Some isolates showed high genetic similarity and were in different HCWs and groups, highlighting the importance of hand hygiene to minimize the risk of cross-contamination in NICUs.

Glossary

NICU, neonatal intensive care units; RAPD, random amplified polymorphic DNA; BrSCOPE, brazilian surveillance and control of pathogens of epidemiologic importance; HCW, healthcare professionals; ABHSs, alcohol-based hand antiseptics; BHI, Brain Heart Infusion; SDA, Sabouraud Dextrose Agar; CFU, colony forming units; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; YPD, Yeast Extract Peptone-Dextrose; PBS, phosphate-buffered saline; RPMI, Roswell Park Memorial Institute; LBP, low biomass production; MBP, moderate biomass production; HBP, high biomass production; LMA, low metabolic activity; MMA, moderate metabolic activity; HMA, high metabolic activity; MIC, minimum inhibitory concentration.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Priscila Guerino Vilela Alves], [Nagela Bernadelli Sousa e Silva], [Meliza Arantes de Souza Bessa], [Gabriel de Oliveira Faria], [Ralciane de Paula Menezes], [Lúcio Borges de Araújo] and [Mário Paulo Amante Penatti]. The first draft of the manuscript was written by [Priscila Guerino Vilela Alves], [Denise Von dolinger de Brito Röder] and [Reginaldo dos Santos Pedroso] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Informed consent

Informed consent was obtained from all study participants.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Federal University of Uberlândia (document 2.173.884/2018).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

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Declaration of competing interest

none.

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Artigo 2 – "Infecções relacionadas à assistência à saúde causadas por *Candida* spp. em neonatos críticos: uma análise das superfícies ambientais".

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

Healthcare-associated infections caused by Candida spp. in critical neonates: a look at environmental surfaces

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

RESUMO

Justificativa e Objetivos: Infecções fúngicas invasivas acarretam elevada morbimortalidade em Unidades de Terapia Intensiva Neonatal (UTIN) e estão acompanhadas de um aumento de isolados resistentes, evidenciando o ambiente hospitalar como fonte primordial de contaminação. Este estudo analisou espécies de *Candida* em neonatos em uma UTIN brasileira, avaliou suas condições clínicas e laboratoriais, caracterizou os isolados e revisou a literatura sobre fontes de contaminação em UTINs e resistência antimicrobiana. **Métodos:** Isolados de *Candida* de recém-nascidos (RN) e do ambiente foram identificados e analisados quanto à resistência antifúngica, fatores de virulência e relação molecular. **Resultados:** Quatro RN apresentaram candidíase invasiva: *C. albicans* (2 RN), *C. glabrata* (1 RN) e *C. parapsilosis* stricto sensu (1 RN). Todos RN eram extremamente prematuros (<29 semanas) e utilizaram algum dispositivo invasivo. Dois isolados clínicos demonstraram resistência, um ao fluconazol (*C. parapsilosis* stricto sensu) e o outro a micafungina (*C. glabrata*). Cinco isolados ambientais foram identificados como *C. parapsilosis* stricto sensu 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* stricto sensu. **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 a 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.*

ABSTRACT

Background and Objectives: Invasive fungal infections entail high morbidity and mortality rates in Neonatal Intensive Care Units (NICU) and are accompanied by an increasing prevalence of resistant isolates, highlighting the hospital environment as the primary source of contamination. This study analyzed *Candida* species in neonates in a Brazilian NICU, evaluated their clinical and laboratory conditions, characterized the isolates, and reviewed literature on sources of contamination in NICUs and antimicrobial resistance. **Methods:** *Candida* isolates from newborns (NB) and the environment were identified and analyzed for antifungal resistance, virulence factors, and molecular relationships. **Results:** Four NBs presented invasive candidiasis: *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 susceptibility dose-dependent to fluconazole. 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 neonates and the NICU environment, 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.

RESUMEN

Justificación y Objetivos: Las infecciones fúngicas invasivas conllevan altas tasas de morbilidad y mortalidad en las Unidades de Cuidados Intensivos Neonatales (UCIN) y están acompañadas por un aumento en la prevalencia de aislamientos resistentes, destacando el ambiente hospitalario como la principal fuente de contaminación. Este estudio analizó las especies de *Candida* en neonatos en una UCIN brasileña, evaluó sus condiciones clínicas y de laboratorio, caracterizó los aislamientos y revisó la literatura sobre las fuentes de contaminación en UCIN y la resistencia antimicrobiana. **Métodos:** Se identificaron y analizaron los aislamientos de *Candida* de recién nacidos (RN) y del ambiente en relación con la resistencia antifúngica, los factores de virulencia y las relaciones moleculares. **Resultados:** Cuatro RN presentaron candidiasis invasiva: *C. albicans* (2 RN), *C. glabrata* (1 RN) y *C. parapsilosis* sensu stricto (1 RN). Todos los RN 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 (NICU) are environments where patients are vulnerable to various types of infections. Invasive fungal infections (IFI) 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* (CNA) species has been described in the literature, and *C. parapsilosis* is among the most prevalent species that cause candidemia worldwide. In Brazil, *C. parapsilosis* represents 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 NICU, linked to increased morbidity and mortality.[1,7] The high incidence of these infections suggests failures in hand hygiene among healthcare professionals and in the hospital environment.[3,9] Studies have shown genetic similarity between *C. parapsilosis* isolates from patients and the NICU environment,[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 NICU.[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, stand out.[3,5,10,11]

The present study aims to analyze the *Candida* species isolated from the bloodstream in newborns from a NICU in Brazil, as well as the isolates from the environment of that unit. Additionally, it seeks to evaluate the clinical and laboratory conditions of newborns with bloodstream infections (BSI) caused by *Candida* species; characterize the isolates through phenotypic and genotypic tests; and finally, conduct a literature review addressing potential sources of environmental contamination in NICU, as well as etiological and antimicrobial resistance trends.

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. Neonates with laboratory confirmation of IC were included, and demographic and epidemiological data were obtained from medical records. These newborns were monitored daily by the National Healthcare Safety Network (NHSN) epidemiological surveillance system[12] from admission to

discharge or death within a period of one year.

Collection of clinical and environmental samples and identification of isolates

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).

Samples from the NICU environment were collected three times a day, at the beginning of each of the three work shifts, 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 chloraphenicol, and on plates with agar chromogenic for Candida (Himedia, Mumbai, India). These were incubated at 35°C for up to 72 h. The 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 the 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 evaluated were fluconazole (Fluoxol, La Paz, Bolivia), amphotericin B (Cristalia, São Paulo, Brazil) and micafungin (Raffo, Buenos Aires, Argentina). The test plates were incubated at 35ºC for 24 h 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, and 90% for amphotericin B.[17,18] The interpretation of the

MIC and the cutoff points for each antifungal followed the guidelines of CLSI documents M59, M60 and M27-S4,[19,20] as well as the criteria established by Pfaller and Diekema (2012).[21]

Assessment of biofilm formation

Biofilm biomass production (0.5% crystal violet) was evaluated according to the protocol by Costa-Orlandi et al. (2014),[22] with modifications. The metabolic activity of the biofilm (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 metabolic activity of biofilm.

The results were classified based on the cutoff 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, OD considered were: low<0.097, moderate=0.097–0.2, high>0.2. Negative controls were wells containing only RPMI broth (Roswell Park Memorial Institute). The tests were performed in quadruplicate and repeated three times independently.

Assessment of extracellular hydrolytic enzymes and hemolytic activity

Assessment of the ability of *Candida* spp. 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 the results were carried out as proposed by Menezes et al. (2019).[25]

Molecular analysis

Analysis of genetic similarity was performed by random amplified polymorphic DNA (RAPD-PCR), the extraction of the genomic DNA of the isolates was carried out from cultures in SDA medium (24 h) at 35ºC. The primer oligonucleotides used were OPA9 (5'-GGGTAACGCC-3'), OPA18 (5'-AGCTGACCGT-3'), OPB11 (5'-GTAGACCCGT-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]

Literature review

In September 2023, a literature search was carried out to analyze invasive infections by *Candida* spp. in neonates in the NICU and the role of the unit environment as a source of these infections. The search covered three databases: PubMed (US National Library of Medicine, National Institutes of Health), VHL (Virtual Health Library), and SciELO (Scientific Electronic Library Online).

The research employed a search strategy that involved the combination of terms (*Candida* AND NICU AND environmental) OR (*Candida* AND newborn/neonate AND environmental AND BSI) OR (*Candida* AND inanimate surfaces AND NICU) OR (*Candida* AND IRAS). Only articles in the English language and fully available in the last 10 years were included, if they addressed the previously mentioned theme. Literature review articles and those involving animal experimentation were excluded.

This research was approved by the Human Research Ethics Committee of the Federal University of Uberlândia (no. 2,173,884/2018; CAAE: 86046318,4,0000,5152) and carried out following the ethical standards and precepts required by the Ministry of Health - Resolutions 466/20 12 - 510/2016 - 580/2018.

RESULTS

Sample characterization

During the study, four newborns (NB) presented IC due to *Candida glabrata* (RN 1), *C. albicans* (RN 2 and RN 3) and *C. parapsilosis stricto sensu* (RN 4). All neonates were male biological sex and extremely premature (<29 weeks) and used at least one invasive device. The average length of stay was 70 days and the newborn with *C. parapsilosis stricto sensu* infection died (Table 1).

229

230 **Table 1**. Clinical characteristics of newborns with *Candida* spp. bloodstream infection in a Neonatal Intensive Care Unit.

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	NA	NA	NA	EAC
Invasive devices (days of use)				
PICC	35	47	18	15
UVC	3	8	NA	NA
MV	55	77	10	8
PN	23	20	14	12
PVC	14	23	NA	NA
Antifungals (days of use)				
Fluconazole				
use prior to infection	16	NA	NA	NA
use after infection	NA	10	22	6
Micafungin				
use prior to infection	NA	NA	NA	NA
use after infection	18	NA	NA	6
Length of hospitalization in the NICU (days)	87	126	50	15
Hospitalization time 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

231 BSI, bloodstream infection; EAC, esophageal atresia correction; NA, no information; NB, newborn; NICU, neonatal intensive care unit; MV, mechanical ventilation; NA, no

232 information; PN, parenteral nutrition; PICC, peripherally inserted central catheter; PVC, peripheral venous catheter; UVC, umbilical venous

233

Five isolates were recovered from environmental samples, all identified as *C. parapsilosis stricto sensu*. 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 2. Phenotypic characteristics (biofilm production and susceptibility to antifungals) of *Candida* species isolated from the environment and bloodstream of neonates in the Neonatal Intensive Care Unit, Uberlândia, Minas Gerais, 2024.

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

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.

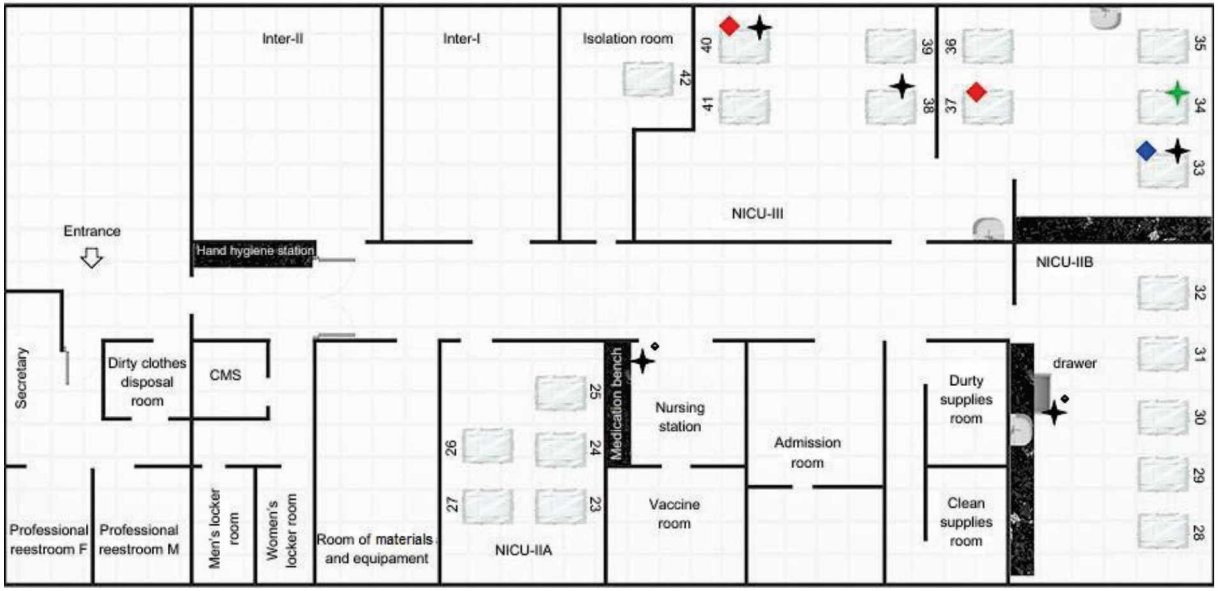

Figure 1. Schematic representation of the Neonatal Intensive Care Unit and location of isolation of environmental and clinical samples included in the study, Uberlândia, Minas Gerais, 2024.Note: *C. albicans* 1 and 2 (red diamonds), *C. glabrata* (blue diamond), and *C. parapsilosis stricto sensu* (green star), all of them *u* from the blood culture, and *C. parapsilosis stricto sensu* from the environment culture (black stars); • identical isolates.

Antifungal susceptibility test

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

Assessment of the production of virulence factors

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 the environment (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).

Determination of genetic similarity of isolates

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

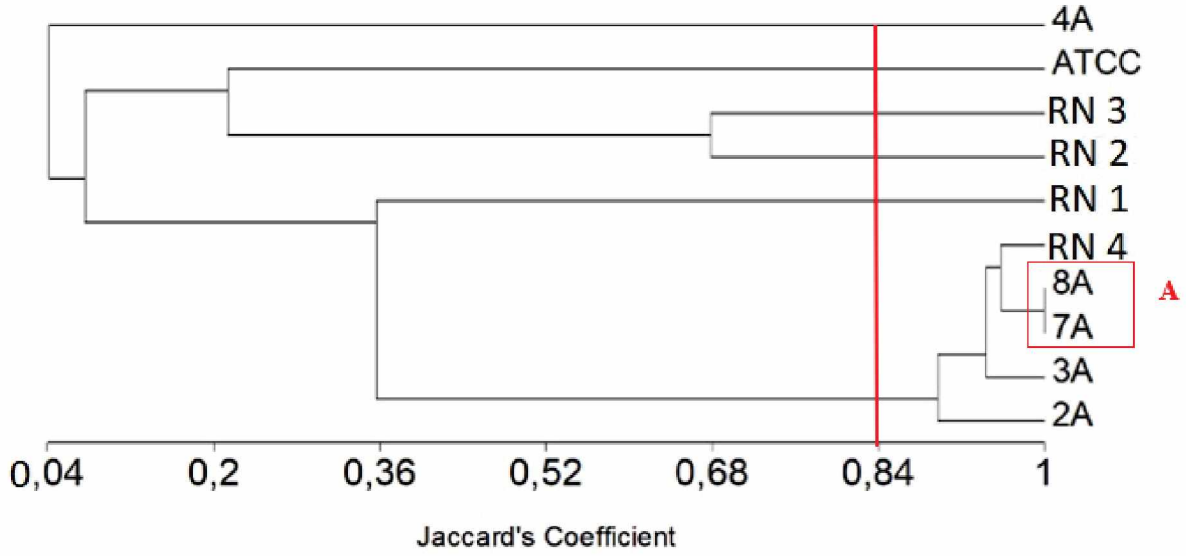

Figure 2. Dendrogram of *Candida* spp. isolates originating from the Neonatal Intensive Care Unit environment and the bloodstream of neonates with combined analysis of primers OPA09, OPA18, OPB11 and OPG17, Uberlândia, Minas Gerais, 2024.

Literature review

Initially, 53 articles were identified in the bibliographic survey. After screening and subsequent exclusion of reviews and duplicate articles, eight were included. Most of the studies (6/8; 75.0%) were conducted in developing countries, and four of them reported the occurrence of outbreaks caused by *Candida* species, Table 3.

Table 3. Data from articles included in the literature review, Uberlândia, Minas Gerais, 2024.

Studies	Clinical isolates			Environment isolates			Genetic similarity	Outcome (n;%)
	Isolation site: species (patients n)	**ASP**	**Virulence factors**	**Isolation site: species (n)**	**ASP**	**Virulence factors**		
[26] Taiwan	Blood: *Candida pelliculosa* (6)	Resistant to amphotericin B (2)	NA	Environment (62): negative	NA	NA	The RAPD patterns of 10 isolates from the six NB were identical to each other	Discharge (5;83.8%) Death (1;16.7%)
[27] India	Blood: *C. krusei* (9)	SDD to fluconazole (1)	NA	Environment: negative	NA	NA	All clinical isolates were from the same clonal origin (FAFLP)	Discharge (7;77.8%) Discharge against medical advice (2;22.2%)
[9] China	Blood: *Candida parapsilosis stricto sensu* (16)	All susceptible	NA	Environment: *C. parapsilosis stricto sensu* (16) Locations: Cleaning cloths, taps, sinks, operating table, puddles of water, fan and ultrasonic probe. Surveillance of the environment: *C. metapsilosis* (23). Locations: Cleaning room sink, negative pressure suction tubes, incubators, nasal mask, ventilator condensate water, incubator humidifying water, disinfectant container, and medical tape.	NA	NA	*C. parapsilosis sensu stricto* isolates from the bloodstream and the environment were identical. *C. metapsilosis* isolates grown from surveillance samples showed similar patterns	Discharge (15;93.8%) Death (1;6.2%)

[28] Brazil	Blood and drain: *C. parapsilosis* sensu stricto (nine samples from six newborns)	All susceptible	Blood: Hemolytic activity (6), DNAse (5) and biofilm (6)	*C. parapsilosis* sensu stricto: seven from the environment (sink (1), doors (2) and incubators (4))	Fluconazole resistant (1; sink)	Hemolytic activity (7) DNAse (5) Biofilm (7)	RAPD: Two identical environmental isolates, (incubators)	Discharge (4;66.7%) Death (2;33.3%)
[29] China	Blood: *C. pelliculosa* (142,017 strains isolated from the blood of 21 NB) Catheter tip: *C. pelliculosa* (7 NB)	SDD to fluconazole (5)	Hyphal cell morphology Biofilm (VC): all produced biofilms	No *C. pelliculosa* was detected in 143 environmental, doctor, and nurse swab samples	NA	NA	qRT -PCR: to confirm the expression of Als4 in *C. pelliculosa* RT-PCR was performed, showing that all isolates express ALS4	Discharge (21;100%)
[30] Egypt	Blood and CSF: *Candida* spp. (27)	NA	NA	Environment: *Candida* spp. (0.6% of 330)	NA	NA	Two strains of *C. tropicalis* were homologous (blood and CSF from two different neonates). Two highly similar strains of *Candida glabrata* (one from the crash cart and one from CSF)	NA
[8] Japan	Blood: *C. parapsilosis* (2)	All susceptible	All formed biofilm	Environment: *C. parapsilosis* (2: incubator and incubator humidifier)	NA	All formed biofilm	All isolates presented the same genotype at the CP1, CP6 and B6 microsatellite loci	NA
[31] India	Blood: Pre-intervention: *C. albicans* (6) Non-*albicans Candida* (22) Post-intervention: *C. albicans* (19) Non-*albicans Candida* (12)	NA	NA	Environment: negative	NA	NA	NA	NA

ASP, antifungal susceptibility; CSF, cerebrospinal fluid; MIC, minimum inhibitory concentration; NA, not applied; NB, newborn; RAPD, Random Amplified Polymorphic DNA; SDD, susceptible dose dependent; VC, violet crystal.

In the studies reviewed, the most of neonates were female (38/70; 54.3%), premature (63/76; 82.9%), and with birth weight between 700 and 2,500 g. The main risk factors for IC included the use of a peripherally inserted central venous catheter (PICC), parenteral nutrition (TPN), broad-spectrum antimicrobials, prematurity, low/very low birth weight, previous or current admission to the NICU, tube, intubation/ventilator, and tracheostomy. Most infections were caused by CNA species, mainly *C. parapsilosis*. The most frequent treatment was fluconazole (38/67; 56.7%) (Miyake, 2023; Qi, 2018; de Paula Menezes, 2020; Zhang, 2021; Hsiao-Chuan, 2013; Elkady, 2022)8,9,26–29). Of the studies that reported on the outcome, they showed that 7.3% (4/55) of affected newborns died (Qi, 2018; de Paula Menezes, 2020, Zhang, 2021; Hsiao-Chuan, 2013; Nagarathnamma, 2017). The species most associated with death was *C. parapsilosis* (3/4; 75.0%) (Qi, 2018; de Paula Menezes, 2020.

Of the eight studies included in this review, half of them (Miyake, 2023; Qi, 2018; de Paula Menezes, 2020; Elkady, 2022identified isolates of *Candida* spp. from environmental samples, with emphasis on objects such as taps, sinks, operating tables, incubators, cleaning cloths and disinfectant containers. The genetic correlation between clinical and environmental isolates was examined in the four studies ((Miyake, 2023; Qi, 2018; de Paula Menezes, 2020; Elkady, 2022), and was found to be positive in three studies (75.0%) (Table 3).

The investigation of virulence factors was addressed in 37.5% (3/8) (Miyake, 2023; de Paula Menezes, 2020; Zhang, 2021) of the articles. These factors included biofilm (3/8; 37.5%) (Miyake, 2023; de Paula Menezes, 2020; Zhang, 2021), DNAse (1/8; 12.5%) (de Paula Menezes, 2020), hemolytic activity (1/8; 12.5%) (de Paula Menezes, 2020) and hyphae formation capacity (1/8; 12.5%) (Zhang, 202127). Regarding the clinical samples tested, all (Elkady, 2022) formed biofilm, eight (27.6%) with high production (Table 3).

DISCUSSION

Invasive candidiasis 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 the hospital environment in the spread of microorganisms, including resistant ones, is recognized,[27] there are few studies on the presence of pathogenic fungi in this context, especially in NICUs. However, studies carried out in countries in Latin America, Africa and Asia have demonstrated the presence of *Candida* spp. in the NICU, highlighting it as a potential source of healthcare-associated infections (HAIs). Therefore, this study seeks to contribute to the understanding of IC and the role of the environment in the persistence and dissemination of these yeasts in the NICU.

In this study we identified three *Candida* species causing IC in the four NB (*C. albicans*, *C. parapsilosis stricto sensu* and *C. glabrata*) and one in the NICU environment (*C. parapsilosis stricto sensu*). The relationship between *C. parapsilosis* infections and the NICU environment indicates the negative impact on newborn 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]

According to the studies reviewed, isolates of *C. parapsilosis* have been identified in the NICU environment.[2-4,8] Menezes et al.[3] reported seven isolates in different points, such as sinks, doors and incubators; Elkady et al.[6] detected *Candida* spp. in 0.6% of the 330 environmental samples analyzed, and Miyake et al.[2] related two isolates, one in the incubator and the other in the incubator humidifier.

In Brazil, some studies have shown a varied distribution of *Candida* species causing IC encompassing bloodstream infections 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 Northern, *C. albicans* led (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 stricto sensu*.[5] This highlights the predominance of non-*albicans Candida* 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, the hospital environment can also be a source, especially in cases of critically ill patients.[14] In our study, *C. parapsilosis stricto sensu* was isolated in the NICU 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 newborns due to the immaturity of the immune system and the fragility of epithelial barriers makes them more prone to IC.[6] All newborns in the study were born at less than 30 weeks of gestation and weighing less than 1,500 g. All newborns in the study were born at less than 30 weeks of gestation and weighing less than 1,500 g. Generally, IC manifests itself around the fourt 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 the ability of this microorganism to remain in the unit for months, suggesting infections through cross-transmission or even intestinal translocation, corroborating our results.[3]

C. parapsilosis is prone to colonizing intravascular catheters and proliferating in individuals using parenteral nutrition.[2,9] The four neonates analyzed in this study used PICC and received parenteral nutrition terapy. One NB had a congenital malformation in the esophagus and was extremely low birth weight (645 g), affected by *C. parapsilosis stricto sensu* 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 the NICU (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 NICU 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 the treatment of IC in NICU 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 NICU.[7] Given the vulnerability of neonates 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 the NICU. 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 neonates and in the NICU environment, 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 the environment as a possible source of infection. These results are in line with findings in the literature, reinforcing the importance of environmental surveillance, rigorous hand hygiene practices and frequent disinfection of the hospital environment, especially in high-touch areas, such as surfaces of incubators.

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Artigo 3 "*Cryptococcus liquefaciens* isolated from the hand of a healthcare professional in a neonatal intensive care unit"

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CLINICAL MICROBIOLOGY - SHORT COMMUNICATION

Cryptococcus liquefaciens isolated from the hand of a healthcare professional in a neonatal intensive care unit

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Abstract

Fungal infections are responsible for high morbidity and mortality in neonatal patients, especially in premature newborns. Infections in neonates caused by *Cryptococcus* spp. are rare, but it has occurred in an immunocompromised population. This study aims to describe the isolation of *Cryptococcus liquefaciens* from the hands of a health professional in a neonatal intensive care unit, and to evaluate the production of biofilm and virulence factors and susceptibility to antifungals. Antifungal susceptibility tests were performed according to Clinical and Laboratory Standard Institute document M27-A3. Thermotolerance virulence factors and DNase, phospholipase, proteinase, and hemolytic activities were verified through phenotypic tests; biofilm was evaluated by determining the metabolic activity and biomass. The isolate did not produce any of the tested enzymes and was susceptible to all antifungals (amphotericin B, fluconazole, and micafungin). The growth at 37 °C was very weak; however, the isolate showed a strong biomass production and low metabolic activity. This is the first report of *C. liquefaciens* isolated from the hands of a health professional. The isolate did not express any of the studied virulence factors in vitro, except for the low growth at 37 °C in the first 48 h, and the strong production of biofilm biomass. *Cryptococcus liquefaciens* can remain in the environment for a long time and is a human pathogen because it tolerates temperature variations. This report draws attention to the circulation of rare species in critical locations, information that may help in a fast and correct diagnosis and, consequently, implementation of an appropriate treatment.

Keywords *Cryptococcus liquefaciens* · Hands · Neonates · Biofilm · Fungal infections · Virulence factors

Priscila Guerino Vilela Alves and Ralciane de Paula Menezes contributed equally to this work.

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Fungal infections are responsible for high morbidity and mortality in neonatal patients, especially premature newborns [1]. Cryptococcosis is an opportunistic fungal infection caused by yeasts of the genus *Cryptococcus* (Vuill., 1901), common not only in human immunodeficiency virus (HIV) patients, but also in organ and stem cell transplant recipients. Although its incidence has rapidly increased in recent years [2], it has rarely been reported in neonates. However, the response to treatment in the context of intrinsic antifungal resistance has become a problem in recent years [3, 4]. The most common pathogenic species are *Cryptococcus* (Vuill., 1901) and *Cryptococcus gattii* (Vanbreus. e Takashio, 2002), while other species are less frequent, as *Cryptococcus albidus* (Saito, 1950), *Cryptococcus laurentii* (Kuff., 1950), and *Cryptococcus liquefaciens* (Saito & M. Ota, 2009) [5]. There are no reports of *C. liquefaciens* in neonates or in critical hospital environments; there have been only two reports in humans [6, 7].

Virulence factors in clinical isolates and infrequent isolates are extensively investigated in an attempt to explain the differences in fungi pathogenicity, as well as to understand the parasite–host relationship, because the presence of these factors facilitates tissue invasion and evasion of mechanism host defense [8].

This study aims to describe the isolation of *C. liquefaciens* from the hands of a health professional in a neonatal intensive care unit (NICU), and to evaluate the production of biofilm, enzymatic activities (DNase, phospholipase, proteinase, and hemolytic), thermotolerance, and susceptibility to antifungals of this isolate.

The collection was carried out at the NICU of a public hospital in Brazil. This hospital unit has 20 beds. *Cryptococcus liquefaciens* was isolated from the hands of a professional who worked at the respective NICU. The study sample was collected following the principles of the "glove bag" technique, with modifications [9]. The identification was performed using matrix-associated laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry. The enzymatic activities (DNase [10], phospholipase [11], proteinase [11], and hemolytic, adapted from Rorig et al. [12]) were performed and interpreted according to the characteristics of each test and were done in duplicate. Interpretation was performed according to Riceto et al. [8].

To evaluate thermotolerance, the sample was inoculated on Sabouraud dextrose agar and incubated at 30 and 37 °C for 48 h. The capsule was observed with China ink.

Biofilm formation and biomass were evaluated by crystal violet 0.5%. Metabolic activity was evaluated with XTT reduction assays (tetrazolium salt, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-(phenylamino)-carbonyl-2H-tetrazolium, hydroxide) (Sigma-Aldrich, St. Louis, MO, USA), performed according to the methodology described by Costa-Orlandi et al. [13] and Pierce et al. [14] (with modifications). The interpretation criteria were those established by Marcos-Zambrano et al. [15]. Readings were obtained from a microplate reader at 570 nm for crystal violet and 495 nm for XTT. The sample was tested in quadruplicate.

Antifungal susceptibility tests to the fluconazole (Fluoxol, La Paz, Bolivia), amphotericin B (Cristalia, São Paulo, Brazil), and micafungin (Raffo, Buenos Aires, Argentina) were performed by the broth microdilution method (MIC), according to Clinical and Laboratory Standard Institute document M27-A3 [16]. The reference strain *Candida parapsilosis* ATCC 22,019 was used for technique control. Tests were performed in duplicates on two different occasions with the sample isolated from the health professional's hand and read at 490 nm with a microplate reader.

The *C. liquefaciens* isolate showed strong biomass production (OD 0.6917) and low metabolic activity (OD 0.2117). The sample incubated at 37 °C showed very low growth and the presence of a capsule (Fig. 1). The isolate was unable to produce any of the exoenzymes and was susceptible to all tested antifungals: amphotericin B (0.25 μg/mL), fluconazole (4 μg/mL), and micafungin (1 μg/mL).

Cryptococcus species are generally isolated from environmental sources and are distributed worldwide, in air, water, wood, soil, and pigeon droppings [17]. In this study, *C. liquefaciens* was isolated from the hands of an NICU health professional. Although this species has not cause fungal infections in the neonates of this unit, it had already been isolated in a previous study of pigeon excreta outside the same hospital in this study [18].

Cryptococcus liquefaciens often colonizes the skin of patients with atopic dermatitis more than the skin of healthy people [19]. Until mid-2015, it was considered non-pathogenic because its capsular components were insufficient to protect against phagocytes [20]. However, there have been reports of disseminated diseases caused by *C. liquefaciens*, one in a 71-year-old patient—isolated from blood culture and catheter tip [6]—and another case of cerebrospinal fluid isolation in a 31-year-old HIV-positive patient, who died [7].

Hospital infections caused by yeast of the genus *Cryptococcus* have already been reported in neonates from Thailand [4], India [2], Kenya [21], and Taiwan [22]. In two studies [2, 22], the isolated strains were susceptible to amphotericin B. Although the isolate from this study was susceptible to all tested antifungals, it is part of the *Cryptococcus* genus, which has been associated with disseminated cryptococcosis in neonates which can more commonly reach the central nervous system, leading to meningitis, encephalitis, obstructive hydrocephalus, chorioretinitis, and endophthalmitis [23]. Susceptibility tests to antifungals are especially important because few antifungals are available for treatment [23]. Due to the limited number of cases, there is no standard validated treatment for *Cryptococcus* infection in neonates [24]. However, in all reported cases, amphotericin B has been the most successful drug treatment [2, 4, 21, 22].

Exoenzymes are considered virulence factors for yeast fungi and can contribute to possible tissue invasion and the pathogenesis of an infectious process [25]. The studied *C. liquefaciens* isolate did not express the investigated exoenzymes when cultured, thus suggesting its probable low virulence.

An ecological strategy that has been associated with chronic infections by *Cryptococcus* spp. is the formation of biofilm. It is estimated that 65% of infectious diseases in humans are related to biofilm formation [26]. Biofilm is a survival strategy mainly used in hostile environments: The production of extracellular matrix protects these microorganisms from potential damage caused by the external environment, allowing them to remain viable for longer on surfaces. These persistent populations are potential reservoirs of microorganisms that can cause chronic and systemic

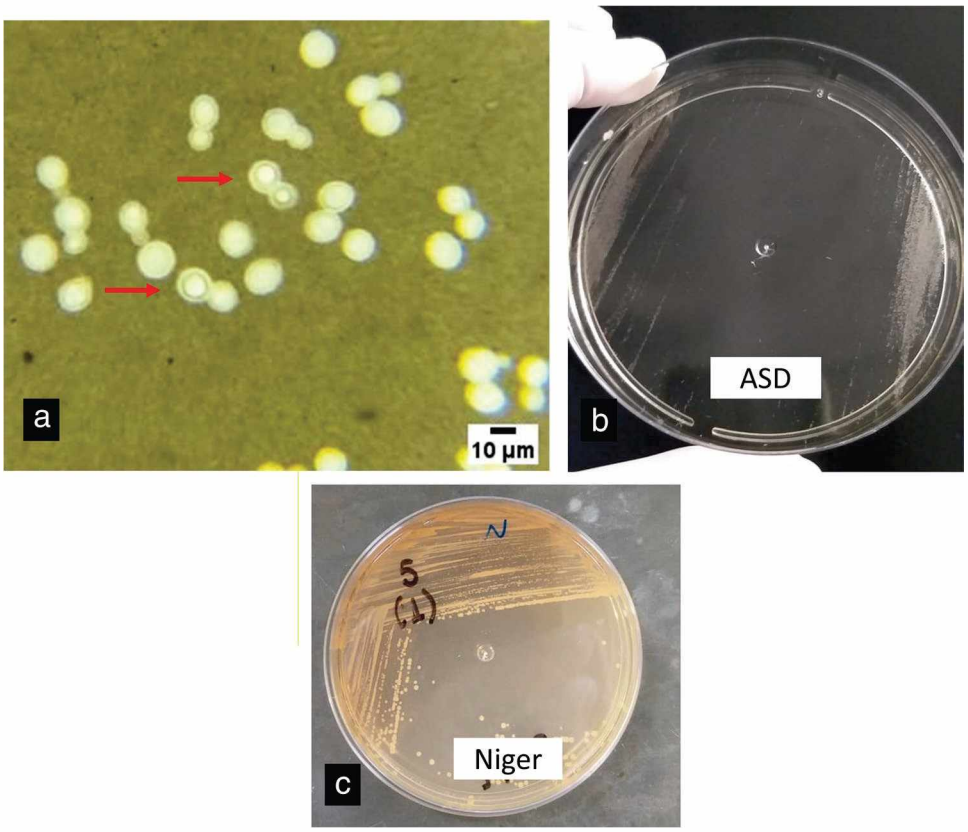

Fig. 1 *Cryptococcus liquefaciens* isolated from the hands of a neonatal intensive care unit health professional. **a** The arrow indicates the presence of a capsule in the sample (400×). **b** Growth of the *C. liquefaciens* sample on Sabouraud agar medium at 37 °C. **c** Growth of the *C. liquefaciens* sample on Niger agar

infections [26]. In the study, a biofilm-producing *C. liquefaciens* isolate was identified in the hands of an NICU health professional; this condition makes these microorganisms 10–1000 times more resistant to antifungal therapy than in their free form [27]. Although the isolate colonized the hands of a healthy person, it was a strong producer of biomass in the biofilm. This factor is relevant not only because of the severity of the infection that this microorganism can cause, but also because the literature has not reported that *Cryptococcus* spp. isolates from the hands have strong biomass production. However, a study by Menezes et al. [28] isolated *Candida* yeast from the hands of health professionals in the same study unit and two isolates were strong biofilm producers.

The World Health Organization considers hand hygiene to be the main form of prevention of healthcare-related infections (HAI) [29]. Health workers are colonized by microorganisms due to the work they perform, and because many of these microorganisms are resistant to antimicrobials and antifungals, health workers start to transmit these agents to patients and other workers. Thus, health workers participate in the epidemiological chain of HAI-related infections [30].

Factors that probably contribute to the increased incidence of human *Cryptococcus* infections (non-*C. neoformans* and non-*C. gattii*) include better laboratory detection, a higher incidence of immunocompromised individuals, and the selective pressure of global warming towards the development of tolerance to human host temperatures [20]. The study sample showed initial growth at 37 °C, demonstrating the ability to multiply and survive at the human host temperature.

This study points to the need for further studies with *C. liquefaciens*, not only in the NICU, but also in other hospital units. Further studies are also needed to verify in vivo the pathogenicity of *C. liquefaciens* in animal models, as well as molecular tests such as sequencing this species.

Cryptococcus species non-*C. neoformans* and non-*C. gattii* are uncommon and are at risk of being identified as environmental contaminants. This is the first report of *C. liquefaciens* isolated from the hands of a health professional. The isolate did not express any of the studied virulence factors in vitro, except for low growth at 37 °C and strong biomass production in biofilm. *Cryptococcus liquefaciens* can remain in the environment for a long time and represents a human pathogen because it tolerates temperature variations. This report draws attention to the circulation of rare species in critical locations, information that may help in a fast and correct diagnosis and, consequently, implementation of an appropriate treatment.

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Author contribution Priscila Guerino Vilela Alves and Ralciane de Paula Menezes conceived the work with the contribution of Denise Von Dolinger de Brito Röder and Reginaldo dos Santos Pedroso. Priscila Guerino Vilela Alves and Murilo de Oliveira Brito collected the sample. Priscila Guerino Vilela Alves, Murilo de Oliveira Brito, Gabriel de Oliveira Faria, Nagela Bernadelli Souza e Silva, and Renner Soares Cruvinel carried out the experiments. Priscila Guerino Vilela Alves and Ralciane de Paula Menezes made inferences and analyzed the results, with the contribution of Denise Von Dolinger de Brito Röder and Reginaldo dos Santos Pedroso. Priscila Guerino Vilela Alves and Ralciane de Paula Menezes wrote the paper and revised the writing with the contribution of Denise Von Dolinger de Brito Röder, Reginaldo dos Santos Pedroso, Mário Paulo Amante Penatti, Gabriel de Oliveira Faria, Nagela Bernadelli Souza e Silva, Murilo de Oliveira Brito, and Renner Soares Cruvinel. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval This study was approved by the Research Ethics Committee of the Federal University of Uberlândia, under no. 2.645.323/2018.

Informed consent The professional was informed about the research, and the collection was only carried out after he/she signed the informed consent form.

Conflict of interest The authors declare no competing interests.

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APÊNDICE 1 – FICHA DE VIGILÂNCIA *"NATHIONAL HEALTHCARE SAFETY NETWORK"* (NHSN)

FICHA DO PACIENTE DA UTI-NEONATAL **UTI-III ( )** **UTI-II ( )**

Leito: _____

RN de:_____REG:_____

NASC:_____PESO:_____SEXO:_____

DATA																						
PROCEDIMENTOS INVASIVOS:																						
CVC Flebotomia																						
CVC Umbilical																						
CVC PICC																						
CVC Intracath																						
Entubação																						
Sonda Vesical																						
Nutriçã o Parente ral																						
Dreno																						

ANTIMICROBIANOS	DATA INIC.	DATA TERM	ANTIMICROBIANOS	DATA INIC	DATA TERM

CULTURAS			

Código	DATA DIAG.	MATERIAL	MICRO-ORGANISMO
1			
2			
3			
4			
5			
6			

CONDIÇÕES CLÍNICAS		
IG:		
APGAR: **1':** **5'**:		
SNAP-II:		
SNAPPE-II:		
Sepse clínica	Data do diagnóstico da sepse clínica:	
Bolsa Rota:		
Tipo de Parto:		

Código do Isolado														
Antibiótico														
Amicacina								Nitrofurantoína						
Ampicilina								Oxacilina						
Benzilpenicilina								Piperacilina/tazobactam						
Cefepime								Rifampicina						
Ceftriaxona								Sulfametoxazol						
Cefalotina								Sulfazotrim						
Ciprofloxacina								Tetraciclina						
Clindamicina								Vancomicina						

Ertapenem									Imipenem							

Eritromicina									Colistina							
Gentamicina									Tigeciclina							
Meropenem									Ampicilina/sulbactam							

Diagnóstico clínico do RN:

☐ **Sistema Respiratório: SMH (síndrome da membrana hialina), SDR (síndrome do desconforto respiratório), Anoxia, Apnéia**

☐ **Sistema Cardiovascular: cardiopatia congênita**

☐ **Sistema Digestivo: atresia de esôfago, gastrosquise, onfalocele, atresia intestinal, anus imperfurado, enterocolite necrotizante, fístula anal**

ANEXO 1 – FOLHA DE APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA – UFU

UFU Comitê de Ética em Pesquisa

UNIVERSIDADE FEDERAL DE UBERLÂNDIA/MG

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Impacto da transmissão cruzada de micro-organismos epidemiologicamente importantes em uma UTI neonatal

Pesquisador: Denise Röder

Área Temática:

Versão: 1

CAAE: 86046318.4.0000.5152

Instituição Proponente: Instituto de Ciências Biomédicas

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.678.162

Apresentação do Projeto:

O projeto em análise é uma projeto observacional prospectivo de vigilância epidemiológica para avaliar a transmissão cruzada de microorganismos no ambiente de UTI neonatal em um hospital universitário.

Objetivo da Pesquisa:

O objetivo principal do estudo segundo os pesquisadores é: "Análise prospectiva da transmissão cruzada por micro-organismos: bactérias gram positivas, gram negativas e leveduras em neonatos internados na Unidade de Terapia Intensiva Neonatal."

Avaliação dos Riscos e Benefícios:

Os autores relatam o risco de identificação mas apresentam medidas para minimiza-lo. O benefício apresentado é o da própria produção dos dados não havendo nenhum para os participantes.

Comentários e Considerações sobre a Pesquisa:

O projeto apresentado apresenta um estudo de vigilância epidemiológica que avalia contaminação por microorganismos no ambiente da UTI neonatal de um hospital universitário. Coletas serão realizadas para