



Detection of fungi in fabric toys for immunocompromised children: an experimental study

Análise das publicações científicas relacionadas à prevenção da hepatite B na última década: 2013-2023

Detección de hongos en juguetes de tela para niños inmunocomprometidos: un estudio experimental

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ABSTRACT

Introduction: Immunosuppression may occur as an adverse reaction to chemotherapy treatment. Thus, neutropenia makes children with cancer susceptible to infectious conditions. In this context, fabric toys bring comfort and coziness, are malleable, and pose no risk of injury, but they can be considered a focus of pathogenic microorganisms. **Aim:** Identifying fungal microorganisms in fabric toys used by patients undergoing cancer treatment. **Outlining:** Samples were collected using swabs soaked in Potato Dextrose (PD) medium directly from the surface of fabric toys, plush toys (B1), and comfort objects (B2), which circulate in hospital and domestic environments of children with oncological diseases. Analysis occurred through molecular identification, conducted by DNA extraction. **Results:** Six (6) fungal isolates of B1 from the genus *Trichoderma* and two (2) fungal isolates of B2 from the species *Aspergillus niger* were obtained. **Implications:** The method used allowed identifying fungal microorganisms present in toys at the genus and species level, reinforcing the idea of finding sanitizers or means to promote safe disinfection of plush toys brought from home to prevent their transmission.

DESCRIPTORS

Oncology; Nursing; Invasive Fungal Infections; Play and Playthings; Neoplasms; Pediatrics.

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INTRODUCTION

Cancer is a set of diseases characterized by the uncontrolled proliferation of abnormal hematopoietic cells, which rapidly spread to tissues and organs. Childhood and juvenile cancer predominantly affect the lymphatic, hematopoietic, and central nervous systems. Additionally, it has a rapid proliferation capacity, necessitating more aggressive treatment.¹

The survival rate is closely related to the early identification of signs and symptoms, the staging of the disease according to clinical history, and treatment management.² Thus, it is noteworthy that most treatments, such as chemotherapy, surgery, radiotherapy, and hematopoietic cell transplantation, have side effects that damage healthy cells and compromise the body's defense system. This makes the child susceptible to opportunistic infections such as Invasive Fungal Diseases, Severe Acute Respiratory Syndrome due to Coronavirus, and infections by multi-resistant bacteria. This situation often requires prolonged hospitalization and interruption of antineoplastic treatment.³⁻⁴

Furthermore, exposure to environments where microorganisms proliferate can lead to infections, given the compromised immune system. Therefore, precautions and restrictions are necessary during antineoplastic treatment, which involves a routine of hospitalizations and procedures. Consequently, being removed from their everyday life brings about feelings of fear, anguish, anxiety, and stress, depriving them of social interaction and typical childhood experiences.⁵

To address this, healthcare professionals have been incorporating practices to assist children in facing this situation, ensuring that play, intrinsic to childhood, is preserved. This is guaranteed by the Universal Declaration of the Rights of the Child adopted by the United Nations General Assembly (1959) and the Child and Adolescent Statute in Brazil (1990).⁶⁻⁷ Toys serve as facilitators of playful activities, contributing to the stimulation of

cognitive, motor, social, and emotional development.⁸

Recreation spaces, such as toy libraries, are intended to stimulate children to play, express themselves, and interact. When implemented in a hospital environment, they aim to alleviate stress during hospitalization, thus contributing to the recovery process.⁹

However, there is a concern among professionals who care for children diagnosed with cancer regarding toys being a vehicle for the transmission of microorganisms. These toys circulate between home and hospital environments and come into contact with mucous membranes, body fluids, and surfaces during play. This is particularly concerning given that these children are immunosuppressed and more susceptible to environmental pathogens.¹⁰

Results of a study have identified microorganisms present in toys, especially those commonly used in hospital toy libraries, which can contribute to nosocomial infections. Since children often do not understand hygiene guidelines and tend to put their hands and toys in their mouths, they are exposed to microorganisms present there.⁹

Accurate identification of fungi in the hospital environment is crucial to ensure the effectiveness of infection control measures and to guarantee patient safety. Several studies have employed the observation of macromorphology and micromorphology as research methods to identify fungi, highlighting characteristics such as coloration, texture, growth patterns, and distinctive microscopic structures. These elements are essential for differentiating species and determining their pathogenic potential. Precise identification of fungi enables targeted and specific therapeutic choices, thus contributing to effective prevention and control of hospital-acquired infections. Additionally, it promotes better environmental management and biosafety practices, including the proper selection of sanitizers and infection control procedures.¹¹⁻¹³

In hospital environments, fabric toys are not recommended due to the rigorous cleaning and disinfection procedures required. These procedures typically involve high temperatures and the use of bleach to eliminate both anaerobic and aerobic microorganisms. However, despite this recommendation, fabric toys are often favored for their comforting texture and flexibility.¹⁴⁻¹⁵

Currently, there are over 100,000 formally described species of fungi, although fewer than 100 are commonly associated with human pathologies. Fungi are found in a variety of habitats, but their presence can be potentially harmful when they occur in environments geared towards human healthcare, such as hospitals. The occurrence of fungi in hospital areas has been reported in several countries, including Brazil. Fungi from the general *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium*, and yeasts of the *Candida* genus are commonly found in various hospital sectors. Although these fungi are considered non-pathogenic, their presence in the hospital environment associated with various mycoses, such as ear infections, urinary tract infections, nail infections, ocular infections, and fungemia. In recent years, there has been a significant increase in the number of infections caused by fungi previously considered non-pathogenic. These infections are often associated with the immunodeficiency that occurs in patients with cancer, HIV/AIDS, and those receiving immunosuppressive treatment.¹⁶⁻¹⁸

Fungi play an important role in hospital infections, some of which can be severe and even fatal. The identification of fungus-infected sites in various hospital sectors is of great importance to healthcare professionals and patients who frequent or remain in these locations. The main transmission routes of fungi are through dust, aerosols, environmental sources (such as soil and vegetation), fomites, and vectors such as insects.¹⁹

Literature review studies emphasize the crucial importance of identifying microorganisms to

promote safety and prevention of invasive fungal infections. Recognizing specific pathogens, such as *Cryptococcus neoformans*, *Candida auris*, *Candida albicans*, and *Aspergillus fumigatus*, is fundamental for directing appropriate treatment and implementing effective control and prevention measures. The precise identification of microorganisms allows for a targeted therapeutic approach, significantly contributing to improving clinical outcomes and reducing the morbidity and mortality associated with these infections, especially in immunocompromised patients. In pediatric settings, conducting this identification, particularly in toys and objects of attachment, has been highlighted as a measure to assist in establishing preventive measures against invasive fungal diseases.²⁰

In this context, fabric toys bring comfort and coziness, are malleable, and do not pose a risk of injury, but they can be considered a focus of pathogenic microorganisms. Therefore, there is a need for the implementation of actions to prevent diseases, especially Invasive Fungal Diseases, to ensure the promotion of quality of life for children undergoing oncological treatment. The aim of this study is the identification of fungal species in fabric toys used by patients undergoing cancer treatment.

METHOD

This is an experimental study conducted with samples and cultures collected from fabric toys brought from home for individual use during the hospitalization of children up to 12 years old, who circulate in hospital and home environments with a diagnosis of cancer at a Pediatric Oncology and Hematology Treatment Center located in Campo Grande, Mato Grosso do Sul, Brazil, in August 2022.

For the collection, children, their belongings and legal guardians were initially identified, who were then invited to participate in the study. To this end, the study and objectives were explained. The Informed Consent Form (ICF) was signed by the legal

guardian, and the Informed Assent Form (IAF) was signed by the child after acceptance.

Two objects were identified: a plush toy (B1) and another attachment object called a "security blanket" (B2). Swabs soaked in Potato Dextrose (PD) medium were passed directly over the entire surface of these objects.

Subsequently, the samples were placed in Falcon tubes and transported to the laboratory for isolation and identification of microorganisms (fungi). Each of the samples was cultured on Petri dishes containing a specific medium for fungi (Sabouraud Dextrose Agar) and incubated at 35°C for 7 days. After fungal growth, colonies were isolated based on morphology and replanted on Sabouraud Dextrose Agar (SDA) plates and incubated at 35°C to obtain the giant colony.

For fungal identification, molecular identification was chosen, performed through DNA extraction. Fungal growth was promoted in liquid Potato Dextrose (PD) medium at 35°C, in an orbital shaker at 150 rpm, for 7 days. Subsequently, mycelial growth was separated from the medium by filtration using a 42mm Whatman filter, and genomic DNA was extracted using the MobioPowersoil® Kit, following the manufacturer's specifications.

DNA amplification was carried out by PCR reaction, and thus the ITS1, 5.8S, and ITS4 regions of rDNA were amplified by PCR technique in a thermocycler (Peltier Thermal Cycler 200, MJ Research). This was programmed to perform an initial denaturation at 94°C for 5 minutes, followed by 24 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 7 minutes.²¹

The primers ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') were used, which are specific to specific positions of the 18S and 28S rDNA. The amplification reaction was performed in a final volume of 50 µL, containing 1x Buffer (50 mM KCl, 20 mM Tris-HCl, pH 8.4), 3.7 mM MgCl₂, 1 mM dNTP, 0.05

U. µL⁻¹ Taq DNA polymerase (Invitrogen), 0.4µM of each primer, and 3ng of DNA. The amplified fragment was served by agarose gel electrophoresis at 1.4% at 3 V.cm⁻¹, along with the 100 bp DNA Ladder molecular weight marker (Invitrogen).

The sample was then sent for sequencing to the company ACT Gene Molecular Analysis Ltd. (Center for Biotechnology, UFRGS, Porto Alegre, RS), which used the AB 3500 Genetic Analyzer automatic sequencer equipped with 50 cm capillaries and POP7 polymer (Applied Biosystems). DNA templates were purified using the ExoSAP-IT™ PCR Product Clean up reagent (Applied Biosystems) and quantified on the Nanodrop 2000 c equipment (Thermo Scientific). They were labeled using 2.5 pmol of specific primer, followed by the phylogenetic analysis process.

The sequences obtained by sequencing were used for phylogenetic composition, performed by the MEGA 11 software,²² where they were compared to type sequences based on the results observed in BLASTn, obtained in the NCBI databases. The phylogenetic tree was built based on the alignment performed by Muscle,²³ subsequently converted into a distance matrix determined by the Kimura-2 parameter,²⁴ which was grouped by the Neighbor-Joining method⁽²⁵⁾. The consistency of the tree structure was determined by bootstrap analysis, based on 1000 sub-samplings in the distance matrix. The fungus *Synchitriumendobioticum* obtained from the National Center for Biotechnology Information (NCBI) database with the accession number OL415114.1 was used as an outgroup, thus obtaining the identification of fungi.

The research project was approved by Research Ethics Committee, report number: 5.781.407.

RESULTS

After isolation, six (6) fungal isolates from B1 and two (2) fungal isolates from B2 were obtained. With the data from sequencing analysis of the ITS1-5.8S-ITS4 region of rDNA, and through BLAST

analysis (<http://www.ncbi.nlm.nih.gov/blast/>) of the

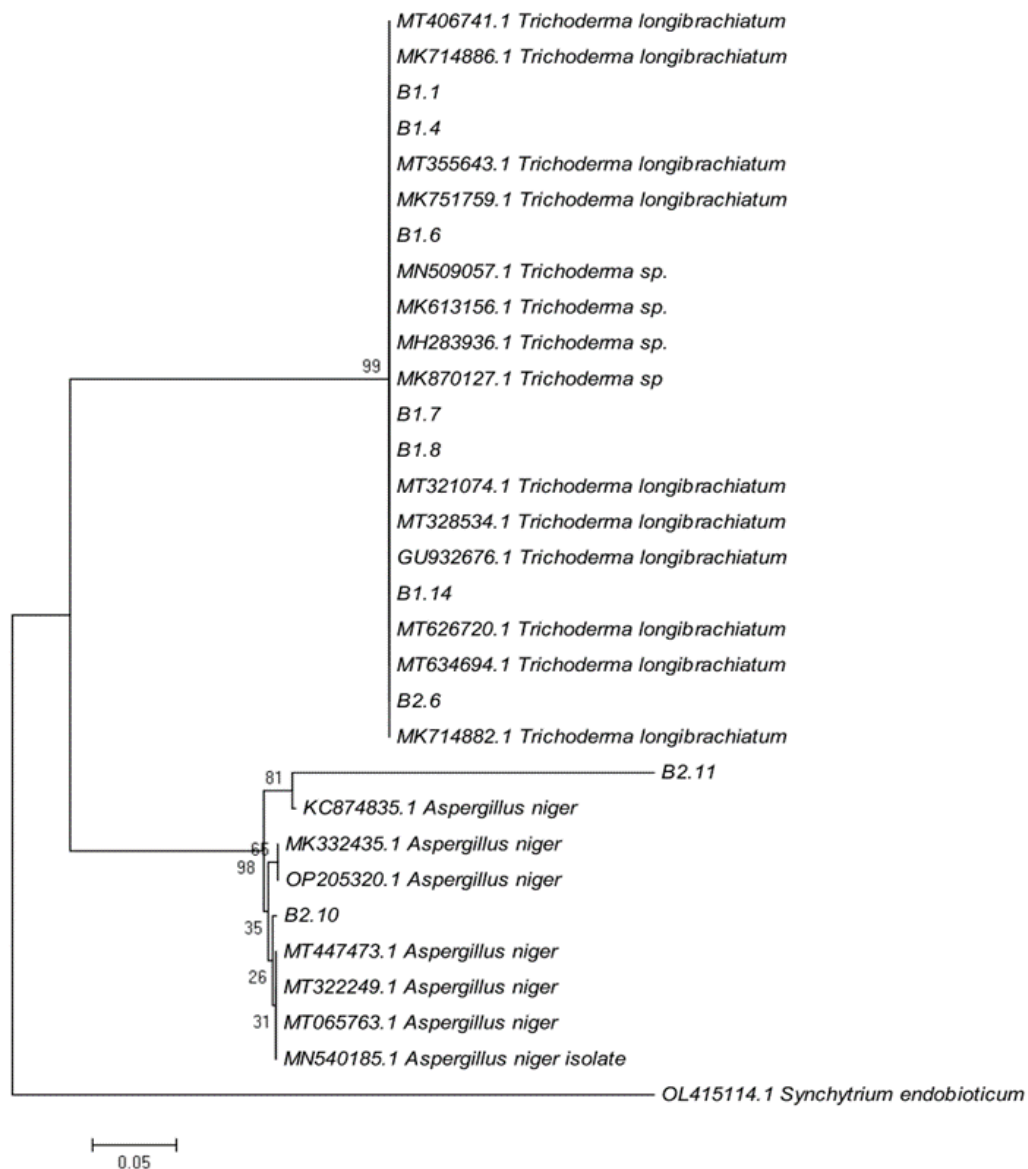
GenBank database, it was possible to identify the nine (9) isolates as described in Table I.

Table 1. Isolated and identified fungi from B1 and B2, and their relationship with the genus or species by percentage of identity found on the NCBI (National Center for Biotechnology Information) website.

Fungal isolate	Object	Closest match on GenBank	GenBank accession number	% Identity
B1.1	B1	<i>Trichoderma longibrachiatum</i>	MT406741.1	99.5%
B1.4	B1	<i>Trichoderma longibrachiatum</i>	MT626720	100%
B1.6	B1	<i>Trichoderma longibrachiatum</i>	MT626720	99.7%
B1.7	B1	<i>Trichoderma sp.</i>	MK870127.1	97.5%
B1.8	B1	<i>Trichoderma longibrachiatum</i>	MT634694	99.6%
B1.14	B1	<i>Trichoderma longibrachiatum</i>	GU932676.1	98.6%
B2.10	B2	<i>Aspergillus niger</i>	MT447473.1	98.4%
B2.11	B2	<i>Aspergillus niger</i>	KC874835.1	84.6%

Source: the authors (2024).

Figure 1. Phylogenetic tree constructed with sequences of the teddy bear and security blanket isolates, and GenBank sequences.



Source: the authors (2024).

The figure represents the phylogenetic tree constructed from sequences of the teddy bear and security blanket isolates, as well as sequences from GenBank (indicated by the database codes). Its construction was performed using the neighbor-joining method and the p-distance matrix for nucleotides, with the option of excluding gaps between pairs. The numbers above and below each node indicate the frequency (%) of each branch in bootstrap analyses with 1000 repetitions.

In this way, it was possible to evidence the presence of fungi from the *genus Trichoderma sp.*, *Aspergillus*, and the species *Aspergillus niger* in the analyzed objects. The data presented show that the fabric toy, even though it is the child's personal use during oncological treatment, carries a potential contamination load for the development of Invasive Fungal Disease. Since immunosuppression associated with frequent exposure to the attachment object may favor the progression of infection, leading to prolonged hospitalization and interruption of antineoplastic treatment for the current health condition.

This fact emphasizes the need for research to develop hygiene and disinfection measures for these fabric toys to ensure the right to play safely during the hospitalization process.

DISCUSSION

The search for identifying microorganisms on toys has been the subject of study by researchers with the aim of improving the composition, formulation, and use of new sanitizers with antifungal action or even their introduction into the composition of toy materials.

In this regard, a study aimed to identify the fungi present in one of the toys highly desired by children and widely promoted on "YouTube" channels: homemade Slime. It is made with glue, dye, glitter, boric acid, and baking soda (without the addition of antifungal). These components drew the attention of researchers regarding fungal growth, mainly because

they are stored anywhere, favoring the growth of microorganisms. Fungal identification was done macroscopically and microscopically, resulting in the identification of the genus *Aspergillus sp* in all samples (five), with the isolates classified as *Aspergillus Flavi* (three isolates) and *Aspergillus Niger* (two isolates).²⁶

Therefore, the authors concluded that the presence of these fungi in slime could be detrimental to children's health and recommended preventive measures such as adding antifungal to homemade slime and close supervision by adults during play to prevent microorganism proliferation and infections. Additionally, there is a need for monitoring by a regulatory body like Anvisa on slime-type toys to ensure safety standards.

Furthermore, another study reinforces the idea of finding sanitizers or means to promote safe disinfection of stuffed toys brought from home to reduce stress during children's hospitalization. Swabs were collected from stuffed teddy bears used by patients in a surgical ward to comfort them during the pre and postoperative period. However, it was concluded that although often seen as comforting for children in stressful situations, they can represent a significant risk of bacterial contamination in the surgical room.²⁷

This suggests that these toys can serve as vehicles for the transmission of infectious agents in hospital environments. Despite parents' perception of the cleanliness and cuddliness of teddy bears, bacteriological results indicate that they do not meet the necessary hygiene standards in a surgical environment. Therefore, considering the infection potential associated with the use of these toys, it is recommended that their presence be reassessed and restricted in surgical rooms to mitigate the risk of surgical site infections.²⁷

Similarly, a study conducted in a childcare center reinforces the idea of toy hygiene, as it identified the presence of microorganisms on 20 toys from schools and daycare centers. Samples were

collected using moistened sterile swabs and then incubated at 37°C for 24 hours on nutrient agar medium. Gram staining technique was used for morphological identification, biochemical identification, and motility test, which only allows the identification of the fungus *Candida albicans*. This suggests that toys can act as disease transmission vehicles, increasing the need to promote regular handwashing among school-age children and their caregivers. Moreover, frequent toy cleaning is crucial to creating a safe and healthy environment for child development.²⁸⁻²⁹

The above-mentioned studies reinforce the idea that the molecular identification method used in this study was a differential. In the literature, classical methods are still widely used, due to their low cost and ease of gender and species identification. However, modern methods, such as the one used in this study, ensure greater accuracy in fungal characterization, which is not possible with traditional classical methods. While species identification based on traditional morphology uses the overall morphology of an organism, modern DNA-based techniques require a small amount of fungal sample. However, modern mycologists have been implementing integrated approaches using both morphological and molecular data.

In the comprehensive approach of traditional and modern fungal analysis methods, fungal cultivation plays an important role. The production of different morphotypes in culture and other accessory structures are important for identification and characterization. Advances in DNA and RNA sequencing technologies are regularly helping researchers to study fungi in an integrated manner and understand their biology, ecology, and taxonomy more effectively.³⁰

The fungus found, *Aspergillus niger* at the species level, has been found in several studies of fungal identification in hospital environments. It morphologically presents with septate hyphae and colonies with dark coloration. They are omnipresent

in the air, soil, and decomposing organic matter and are associated with cases of respiratory system involvement due to colonization and destruction of lung tissue by inhalation of conidia by immunocompromised individuals, as well as due to hospital renovations and constructions.³¹

Another difference from other studies was the identification of the fungus of the genus *Trichoderma spp*, which has more than 200 species and morphologically presents with coenocytic hyphae, greenish coloration, rapid growth of colonies, and asexual reproduction. As they have potential in biocontrol of phytopathogens, regulating plant growth rates, and inducing resistance to stressful agents, the number of agricultural products based on these fungi has increased in recent years, with an increase in dissemination in the environment.³²

These findings reinforce the idea of the importance of using sanitizers that remove *Trichoderma sp* fungi from surfaces. Although less common, a study found it to be the main etiological agent for invasive lung infection in neutropenic patients undergoing treatment for Acute Myeloid Leukemia, along with *Aspergillus sp*. For this case, antifungal therapy with voriconazole was initiated, in addition to a combination of caspofungin-voriconazole for 10 days, followed by oral monotherapy with voriconazole for 8 weeks for the resolution of Invasive Fungal Disease.³³ This demonstrates that besides being difficult to control, treatment also burdens healthcare services. A study conducted in the United States showed that hospitalized patients with Invasive Fungal Disease generate a total cost of \$4.6 billion, with *Aspergillus sp* responsible for 14,820 hospitalizations and a total cost of \$1.2 billion.³⁴

Another important issue regarding the contamination of immunocompromised patients by fungi during antineoplastic therapy is that depending on the class of antifungal, there may be pharmacological interaction due to absorption or effects on CYP450 cytochrome enzymes, which can

lead to the risk of toxicity and the need for prolonged treatment.³⁵

A major concern related to pharmacological therapy has been the resistance shown by some types of fungi, such as *Aspergillus sp*, due to mutations in the gene associated with 14-demethylase (*cyp51*), which encodes the target enzyme of these fungi.³⁶

Thus, the importance of preventing Invasive Fungal Disease in pediatric patients undergoing oncological treatment has been increasingly discussed, aligning with this study that proposes the identification of fungi from children's personal use objects to facilitate the choice of sanitizers and emphasize the importance of toy hygiene routines.

The identification of fungi on toys is necessary to promote proper cleaning and disinfection of toys in pediatric environments, emphasizing the importance of appropriate sanitizers. They highlight the need for effective methods to reduce the risk of disease transmission in places such as daycare centers, pediatric hospitals, and waiting rooms of general clinics. Research examines different approaches, from the use of specific disinfectants to regular toy washing, aiming to eliminate pathogenic microorganisms. Additionally, they emphasize the relevance of proper cleaning protocols and the correct use of products such as 70% alcohol and recommended disinfectant solutions. These measures are crucial to protect children's health, especially in environments where contamination may be more prevalent or when there are immunosuppressed children.³⁷⁻³⁹

Limitations

This study has limitations. The sample size and the data collection location at a single hospital affect the generalizability of the results. These factors may not reflect the diversity of fungal microorganisms present in toys from other hospitals or regions with different climatic, sanitary, and hospital protocol conditions. This geographic limitation could influence the variability of the

identified microorganisms, restricting the applicability of the findings to other populations and pediatric contexts.

Additionally, the small sample size may not capture the extent of fungal contamination in personal use toys in a hospital setting, potentially underestimating or overestimating the prevalence of certain genera and species of fungi. It is recommended that future studies be conducted with larger and more diverse samples, encompassing various materials and collection sites, to validate and expand the results, providing a more robust basis for implementing hygiene and disinfection practices for toys in hospital environments.

Implications for Practice

The findings of this research highlight the importance of studies focused on identifying safe and effective cleaning methods for fabric toys to prevent the spread of pathogens. This, in turn, can contribute to reducing hospitalization time, associated costs, and ensuring better patient prognosis. Additionally, it is crucial to emphasize the need for further research on appropriate hygiene and disinfection practices for these toys, ensuring that the material is not damaged nor the characteristics of the object altered. The findings aim to assist in developing toy hygiene protocols to maintain children's right to play, considering that toys help reduce fear and pain during hospitalization, thus aiding in coping with the illness.

CONCLUSION

The method used in this study allowed for the identification of fungal microorganisms present in toys at the genus and species level. This technique ensured more accurate results which were fundamental in relating them to Invasive Fungal Diseases that affect immunocompromised children.

In addition, these findings point to the emergence of new studies aimed at finding safe and effective means of cleaning fabric toys to prevent

pathogens, and consequently contribute to reduction of hospitalization time, costs and ensure better patient prognosis.

It should also be emphasized that there is a need for further study regarding the adequate hygiene and disinfection of these fabric toys, without damage to the material and without changing the object, to ensure playing without risks and restrictions, considering that toys contribute to

reducing fear and pain during hospitalization and favor coping with the disease.

Thus, although maintaining a child's toy brings comfort to them during hospitalization, it is essential that there is adequate handling and care, so that these objects are used safely and do not become a means of transmitting Invasive Fungal Diseases to the patient during the period of immunosuppression.

RESUMO

Introdução: A imunossupressão pode ocorrer como uma reação adversa ao tratamento quimioterápico. Portanto, a neutropenia torna as crianças com câncer suscetíveis a condições infecciosas. Nesse contexto, os brinquedos de tecido trazem conforto e aconchego, são maleáveis e não representam risco de lesões, mas podem ser considerados um foco de microrganismos patogênicos. **Objetivo:** Identificar os microrganismos fúngicos em brinquedos de tecido usados por pacientes em tratamento contra o câncer. **Delineamento:** As amostras foram coletadas por meio de swabs embebidos em meio de Batata Dextrose (BD) diretamente da superfície de brinquedos de tecido, brinquedos de pelúcia (B1) e objetos de conforto (B2), que circulam em ambientes hospitalares e domésticos de crianças com doenças oncológicas. A Análise ocorreu por meio de identificação molecular, sendo feita por meio da extração de DNA. **Resultados:** foram obtidos seis (6) isolados fúngicos de B1 do gênero *Trichoderma* e dois (2) isolados fúngicos de B2 da espécie *Aspergillus niger*. **Implicações:** Portanto, o método utilizado permitiu identificar microrganismos fúngicos presentes nos brinquedos em nível de gênero e espécie, reforça a ideia de encontrar saneantes ou meios para promover uma desinfecção segura dos brinquedos de pelúcia trazidos de casa para evitar sua veiculação.

DESCRITORES

Oncologia; Enfermagem; Infecções Fúngicas Invasivas; Brinquedos; Neoplasias; Pediatria.

RESUMEN

Introducción: La inmunosupresión puede ocurrir como una reacción adversa al tratamiento quimioterápico. Así, la neutropenia hace que los niños con cáncer sean susceptibles a condiciones infecciosas. En este contexto, los juguetes de tela brindan comodidad y bienestar, son maleables y no presentan riesgo de lesión, pero pueden ser considerados focos de microrganismos patogênicos. **Objetivo:** Identificar microrganismos fúngicos en juguetes de tela utilizados por pacientes en tratamiento contra el cáncer. **Delineación:** Se recogieron muestras utilizando hisopos empapados en medio Potato Dextrose (PD) directamente de la superficie de juguetes de tela, juguetes de peluche (B1) y objetos de consuelo (B2), que circulan en ambientes hospitalarios y domésticos de niños con enfermedades oncológicas. El análisis se realizó mediante identificación molecular, llevada a cabo mediante extracción de ADN. **Resultados:** Se obtuvieron seis (6) aislados fúngicos de B1 del género *Trichoderma* y dos (2) aislados fúngicos de B2 de la especie *Aspergillus niger*. **Implicaciones:** El método utilizado permitió identificar microrganismos fúngicos presentes en los juguetes a nivel de género y especie, reforzando la idea de encontrar desinfectantes o métodos para promover la desinfección segura de los juguetes de peluche traídos de casa para prevenir su transmisión.

DESCRIPTORES

Oncología; Enfermería; Infecciones Fúngicas Invasivas; Juguetes; Neoplasias; Pediatría.

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COLLABORATIONS

FRBM and CMEC: substantial contributions to the study design. BSM, LELC, TTA and FRBM: contributions to data collection. BSM, TTA and FRBM: contribution to data analysis and interpretation. BSM, TTA, LMAM, MAM, RGSA and FRBM: contributions to the discussion of results. BSM, TTA, LMAM, MAM, RGSA, FRBM: contributions to the writing and/or critical review of the content and approval of the final version. CMEC, ARR and CEdeO: resources. **All authors agree and are responsible for the content of this version of the manuscript to be published.**

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AVAILABILITY OF DATA

Data supporting the reported results are openly available in Figshare at: <https://doi.org/10.6084/m9.figshare.26046334>

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.