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REVIEW ARTICLE

Occurrence of Acanthamoeba in hospitals: a literature review

Ocorrência de Acanthamoeba em hospitais: uma revisão da literatura
Ocurrencia de Acanthamoeba en hospitales: una revisión de la literature

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ABSTRACT

Background and Objectives: *Acanthamoeba* are among the most prevalent environmental protozoans and have a cosmopolitan distribution. The main concern in public health is that they can also be isolated from contact lenses, storage cases and cleaning solutions, ventilation and air conditioning systems, dental treatment units, dialysis units, emergency showers and eyewash stations. As these genera include a diversity of pathogenic microorganisms that leads to infectious diseases inside the hospital environment, it is of the utmost importance to carry out the surveillance, considering mainly the immunocompromised patients, who are more susceptible to these diseases. The level of human health risk and its associations in the hospital environment are unknown, and part of this problem is potentially the lack of correlation between protozoan exposure and the onset of symptoms that can occur in each patient at different periods in time. Thus, this review offers a current overview of the presence of *Acanthamoeba* spp. in hospital environments with the aim to detect its presence in these environments. **Methods:** This is a review of the literature on Lilacs, Scielo, Medline and Bdenf databases to gather and synthesize publications and search for effective ways of controlling the presence of *Acanthamoeba* through disinfection and monitoring measures. **Results:** We found that *Acanthamoeba* is present in different hospital environments, namely in water, dust, biofilm, cooling waters and air conditioners. **Conclusion:** Studies on the ecology and distribution of non-enteric pathogens in the hospital environment are necessary for understanding their potential threat to human health.
Keywords: *Acanthamoeba spp., hospital, environment.*

RESUMO

Justificativa e objetivos: *Acanthamoeba* estão entre os protozoários ambientais mais prevalentes e possuem distribuição cosmopolita. A principal preocupação para fins de saúde pública é que eles também podem ser isolados de lentes de contato, caixas de armazenamento e soluções de limpeza, sistemas de ventilação e ar condicionado, unidades de tratamento odontológico, unidades de diálise, chuveiros de emergência e lava-olhos. O gênero *Acan-*

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thamoeba compreende várias espécies que podem determinar doenças infecciosas dentro do ambiente hospitalar, e é de suma importância realizar a vigilância, considerando principalmente os pacientes imunocomprometidos, que são mais suscetíveis a essas doenças. O nível de risco para a saúde humana e suas associações no ambiente hospitalar é desconhecido, e parte desse problema ocorre pela falta de correlação entre a exposição ao protozoário e o início dos sintomas, que cada paciente pode desenvolver em diferentes períodos. Assim, esta revisão apresenta uma visão geral atual de *Acanthamoeba* spp. em ambientes hospitalares, com o objetivo de verificar sua presença nesses ambientes. **Métodos:** Trata-se de uma revisão da literatura nas bases Lilacs, Scielo, Medline e Bdenf para reunir e sintetizar publicações e buscar formas eficazes de controle de sua presença por meio de medidas de desinfecção e monitoramento. **Resultados:** Verificamos que *Acanthamoeba* está presente em diferentes ambientes hospitalares, estando presente em água, poeira, biofilme, águas de refrigeração e ar condicionados. **Conclusão:** Estudos sobre a ecologia e distribuição de patógenos não-entéricos no ambiente hospitalar são necessários para entender sua ameaça potencial à saúde humana.
Palavras chaves: *Acanthamoeba spp, hospital, ambiente*

RESUMEN

Justificativa y Objetivos: *Acanthamoeba* están entre los protozoos ambientales más prevalentes y poseen una distribución cosmopolita. La principal preocupación por la salud pública es que también pueden aislarse de lentes de contacto, cajas de almacenamiento y soluciones de limpieza, sistemas de ventilación y aire acondicionado, unidades de tratamiento odontológico, unidades de diálisis, duchas de emergencia y lava- los ojos. Estos géneros incluyen una diversidad de microorganismos patógenos que conducen a enfermedades infecciosas dentro del ambiente hospitalario, y es de suma importancia realizar la vigilancia, considerando principalmente a los pacientes inmunocomprometidos, que son más susceptibles a esas enfermedades. El nivel de riesgo para la salud humana y sus asociaciones en el ambiente hospitalario es desconocido, y parte de este problema es potencialmente la falta de correlación entre la exposición al protozoario y el inicio de los síntomas, que cada paciente puede desarrollar en diferentes períodos en el tiempo. Así, esta revisión presenta una visión general actual de la presencia de *Acanthamoeba* spp. en ambientes hospitalarios, con el objetivo de verificar su presencia en esos ambientes. **Métodos**: Se trata de una revisión de la literatura en las bases Lilacs, Scielo, Medline y Bdenf para reunir y sintetizar publicaciones y buscar formas eficaces de control de su presencia por medio de medidas de desinfección y monitoreo. **Resultados**: En el presente trabajo se analizan los resultados de la evaluación de la calidad de los alimentos y de los productos alimenticios en el medio ambiente hospitalario, que se encuentran en el agua, polvo, biofilm, aguas de refrigeración y aire acondicionados. **Conclusión**: Estudios sobre la ecología y distribución de patógenos no entéricos en el ambiente hospitalario son necesarios para entender su amenaza potencial salud humana.
Palabras clave: *Acanthamoeba spp, hospitalario, ambiente.*

INTRODUCTION

Acanthamoeba are among the most prevalent environmental protozoans. They have a cosmopolitan distribution and have already been isolated in the most distinct natural habitats including soil, dust, air and beach sand.[1] They have also been identified in aquatic environments, such as sea water, swimming pools, thermal waters and sewage treatment systems.[2,3] The main concern for public health purposes is that they can also be isolated from contact lenses, storage cases and cleaning solutions, ventilation and air conditioning systems, dental treatment units, dialysis units, emergency showers and eyewash stations.[4] They have also been isolated as contaminants in cell cultures of mammalian, bacterial and yeast cells.[5]

Over the past several decades, these organisms have gained increasing attention due to their diverse roles in the ecosystem and in particular, their role as causative agents in severe, and sometimes fatal, human infections.[6] The first cases that clearly established *Acanthamoeba* as the causative agents of disease in humans were reported in the early 1970s. This genus causes three main types of diseases involving the eye (*Acanthamoeba* keratitis), the brain and spinal cord (granulomatous encephalitis), and infections that can spread throughout the entire body (disseminated infection). Individuals who develop granulomatous amoebic encephalitis (GAE) or disseminated disease are usually immunocompromised, whereas those with keratitis are usually immunocompetent. Disseminated disease and GAE carry a poor prognosis, and treatment strategies are not well defined; *Acanthamoeba* keratitis is a sight-threatening infection that carries a favorable prognosis when diagnosed and treated early.[7]

Acanthamoeba spp. during the trophozoite stage, which is the metabolically active stage, feeds on bacteria, fungi and algae by phagocytosis.[8] During this process, microorganisms such as bacteria and fungi normally undergo digestion in the amoebic phagolysosome.[9] Some have evolved and become resistant to these protozoans, able to survive and, in many cases, multiply inside the amoeba until they are released again through the lysis

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of this protozoan or by means of vesicles. These microorganisms are called amoeba-resistant microorganisms (ARM).[10] Several bacteria, fungi, protozoans and pathogenic viruses have been described as ARM.[11-13]

The presence of *Acanthamoeba* spp. in a hospital environment demonstrates that the disinfection measures used are insufficient to remove these protozoans. Their intrinsic resistance to high-level disinfectants highlights the need to better evaluate and understand the actions of these treatments against these "Trojan horses". Therefore, it is necessary to warn health professionals to pay more attention to disinfection processes used in the hospital environment.[14]

If the *Acanthamoeba* spp. can host a diversity of pathogenic microorganisms that lead to infectious diseases inside the hospital environment, it is of the utmost importance to carry out the surveillance, considering mainly the immunocompromised patients, who are more susceptible to these diseases. The level of human health risk and its associations in the hospital environment are unknown, and part of this problem is potentially the lack of correlation between protozoan exposure and the symptom onset, which each patient can develop at different periods in time. Studies on the ecology and distribution of non-enteric pathogens in the hospital environment are necessary to understand their potential threat to human health. Yet one of the biggest challenges remains the effective monitoring. Thus, this review presents a current overview of the presence of *Acanthamoeba* spp. in hospital settings and attempts to provide guidelines on how to deal with their presence in these environments.

Association between *Acanthamoeba* spp. and other pathogens

Acanthamoeba spp. constitute an environmental reservoir and vector for a wide variety of microorganisms, such as bacterial pathogens, fungi and viruses. Among them, the bacteria are the most often documented kind of endosymbiont. Many bacteria are resistant to these amoebae, including *Chlamydia* spp., *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., *Mycobacterium* spp. and *Streptococcus* spp., which makes them completely resistant to water treatment systems and disinfectants used in hospitals.[15] The term "Trojan horse" has been used to name free-living amoebae (FLA) that function as reservoirs for multiplication of other intracellular microorganisms, thus constituting vehicles for pathogen dissemination.[12]

This amoeba internalizes bacteria, for instance, and then groups them into a vacuole, upon fusion with the lysosome, forming a phagolysosome. The acidic pH and lysosomal enzymes lyse the phagocytized bacteria. This ability suggests that amoebae can be used as a model for interaction studies of bacteria with phagocytic cells in humans. However, the virulence determinants for infection and multiplication in human cells and in amoebae phagocytes do not seem to be the same.[6]

Some studies have shown that some amoeba-resistant bacteria are able to alter the phagosome environment few minutes after the internalization, such as *Legionella pneumophila* and *Pseudomonas aeruginosa*. They modify the phagosome traffic, averting the entrance in the host's endocytic pathway and preventing the immediate phagosome-lysosome fusion through the construction of a distinct niche that allows the intracellular bacterial replication.[16,17]

While non-pathogenic bacteria are internalized, killed and used as a source of nutrients, pathogenic ones modify the *Acanthamoeba* spp. intracellular mechanism, ensuring their survival and multiplication after being released through vesicles or by amoeba lysis. Some bacteria do not multiply within the amoeba, but stay in a dormant state, and after being released into the environment, they return to their normal state.[6]

To avoid digestion by amoebae, bacteria can express different sets of genes that are responsible for different intracellular microenvironments and facilitate their survival and growth. Proteins of which expression is regulated within the intracellular compartments may constitute their potential virulence factor. Bacterial secretion systems play a crucial role by providing the bacterial factors involved in these processes to their sites of action. *P. aeruginosa*, for instance, produces various enzymes and toxins released by different secretion systems. The type III secretion system allows the toxin release into cells by bacteria, thereby inhibiting phagocytosis.[17]

Hospitalized patients requiring the use of hospital devices such as endotracheal tubes and catheters are susceptible to *Acanthamoeba* spp. infections with endosymbionts, since these can adhere to biofilms, causing lysis and proliferation and the survival of bacteria inside them. Currently, this association of multiplying endosymbionts is often reported and known to cause extremely severe infections, such as pneumonia or even tuberculosis.[18]

Acanthamoeba spp. ecology in the hospital environment

Due to the opportunistic nature of *Acanthamoeba* and its possible role as a reservoir for human pathogens, monitoring the presence of this protozoan in settings such as hospitals and health facilities, where people are more debilitated and susceptible to infection, is vital.[19] Current investigations on the presence of *Acanthamoeba* in hospital settings are scarce, but the most important ones will be described here.

Water systems or wetlands

The coexistence of FLA and bacteria in hospital water systems was observed in South Africa. The samples were cultured and PCR and DNA sequencing molecular techniques were applied. They identified FLA belonging to the genus *Vermamoeba* spp., *Acanthamoeba* spp. and *Naegleria gruberi*. In the first hospital analyzed, the greatest diversity of bacteria found concomitantly with FLA was observed in the neonatal ward, especially *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Pseudomonas luteola*, *Rhizobium radiobacter* and *Achromobacter denitrificans*. In the second hospital analyzed, the presence of *Pseudomonas*

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and *Staphylococcus* was found in the isolated FLA.[11]

To evaluate the prevalence of FLA associated with endosymbionts in Austria, three different cooling towers of a hospital, tap water and shower were analyzed over a 1-year period. They used PCR to identify the presence of *Acanthamoeba* spp., *Naegleria* spp., *Paravahlkampfia* spp., *Vahlkampfia* spp., *Singhamoeba* spp., *Willaertia* spp., *Tetramitus* spp., *Vermamoeba vermiformis*.[12] The FISH (fluorescence in situ hybridization) technique was used to identify the endosymbionts. The identification of endosymbionts was performed by 16S rDNA gene sequencing. Among the positive samples for FLA, *Acanthamoeba* spp. were the most prevalent ones. The identified endosymbionts were *Paracaedibacter acanthamoebae*, *Legionella rubrilucens* and *L.pneumophila*.[13]

A study based on the biodiversity of amoebae and amoebae-resistant bacteria was performed in a hospital water network. The authors collected water samples, tap and shower swabs from the intensive care unit (ICU), surgical unit and medical clinic ward, and used PCR and 18S rRNA sequencing as the methodology. An *Acanthamoeba polyphaga* strain was isolated from a tap water swab and survived at temperatures of 44° and 47°C but did not show growth at this temperature range. *Mycobacteria* showed a higher prevalence, showing that they are directly associated with FLA in the water networks. In that study, it was demonstrated that *Mycobacteria* can grow in amebae co-cultures *in vitro*, and this allowed the isolation of a new species, the *Mycobacterium massiliense*, from a patient's sputum sample.[19]

Trabelsi[8] and coworkers collected water samples during a four-month period from different wards of the Sfax University Hospital (surgical services, ICU, operating room and water storage tanks). Free-living amoebae were detected in 53.5 % of collected samples, of which *Acanthamoeba* were the most prevalent. These isolates belong to T4, T10, and T11 genotypes and they describe the first report of the T10 and T11 genotype in Tunisia.

Khurana[20] evaluated the extent of FLA contamination in water sources of the ICU, bone marrow transplantation unit, transplant ICU, hemodialysis unit and high dependency unit in a tertiary hospital in India. They confirmed the presence of *Acanthamoeba* spp. genotypes T3 and T4 by PCR and DNA sequencing.

Bagheri[18] performed a study in hospitals of 13 cities in Iran. Samples were collected from hot and cold water taps from different hospital wards. Confirmation of the existence of *Acanthamoeba* spp. was performed using a reverse phase microscopy technique. Based on morphological characteristics, the presence of *Acanthamoeba* spp. was present in practically half of samples collected at temperatures between 21° C and 48° C, showing a significant presence of these microorganisms in hospitals where the water source is the water treatment network.

'Dry' or 'Moist' biofilms

A study by Fukumoto[13] evaluated the coexistence of *Chlamydia* spp. and *Acanthamoeba* spp., and the *Chlamydia* spp. pathogenicity. Smear samples were collected from the floor or sink outlets from different hospital environments during the winter and summer seasons in Japan. The isolated samples were cultured and genetic and phylogenetic analyses were performed. The presence of *Acanthamoeba* spp. was observed in 76.7% of samples, three of which contained *Chlamydia* spp. and only one was potentially pathogenic with ample capacity for infection and proliferation. The prevalence seemed to increase in the summer trial, although without statistical significance, potentially indicating a seasonal variation. Meanwhile, there was no difference in prevalence between swabs from either 'Dry' or 'Moist' conditions or between floors.

Fukumoto[13] randomly collected samples from the floor, sinks and collector's exits of the air-conditioning from a hospital at Hokkaido University, Japan. They used the PCR technique and identified that *Parachlamydia acanthamoeba*, an intracellular bacterium that infects FLA, is considered highly pathogenic in humans with hospital pneumonia and has great implications for the prevention and control of nosocomial infections. The association between *P. acanthamoeba* and *Acanthamoeba* spp. has a significant effect on the long-term survival of the bacterium and increases its performance by spreading in the hospital environment. The authors demonstrated *in vitro* that without the presence of *Acanthamoeba* spp. the survival of *P. acanthamoeba* does not exceed the period of three days at a temperature of 30°C or 15 days at 15°C.

Dust

In a characterization study of *Acanthamoeba* spp., Costa[21] collected dust samples from a public hospital in the city of Curitiba, state of Paraná, Brazil. Samples were collected from five different hospital areas. Morphological analyses of cysts and trophozoites confirmed that they belonged to the group I and group II *Acanthamoeba* spp. genus. The characteristics resemble those of *A. astronyxis* and *A. triangularis* species, although this identification by morphological criteria was not reliable, since the morphology may vary due to factors such as cultivation and condition of the samples. They applied the PCR technique and DNA sequencing analysis. Isolates were identified as *Acanthamoeba* by PCR in samples that belonged to groups I and II. The authors emphasized that the samples belonging to group II are more pathogenic and include several species usually associated with clinical conditions.

Da Silva and da Rosa[22] evaluated the occurrence of *Acanthamoeba* and *Naegleria* genera in FLA in dust samples from two hospitals in the countryside of São Paulo. Amoeba of the genera *Acanthamoeba* and *Naegleria* were found in 45.5% of the samples, of which 41.6% were collected at the university hospital and 50% at the state hospital. Overall, 45.5% were positive for the genus *Acanthamoeba* and 3.8% for the genera *Naegleria*.

Water and biofilm

Muchesa[11] carried out a study on the occurrence of FLA in a teaching hospital in Johannesburg, South Africa. They collected water and biofilm samples from the

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sterilizator, sterilization service unit, central sterilization service unit and endoscopy/bronchoscopy unit. Samples of tap water, dry swabs and shower water were collected. Approximately 90% of samples were positive for FLA; *Acanthamoeba* spp., *Balamuthia* spp. and *Hartmannella* spp. were identified by morphological analysis. The presence of FLA in the hospital water network may be a potential health risk.

Analyzing samples collected at a medical center in the United States, Ovrutsky[15] isolated FLA and Nontuberculous Mycobacteria (NTM). Water and biofilms samples were collected from showerheads and faucets in patients' rooms, drinking fountains, the hospital therapy pool, and disinfection units used to sterilize bronchoscopes and endoscopes. Free-living amoebae were recovered from most of the biofilm samples and the highest prevalence was of the *Acanthamoeba* spp. genus. These results were confirmed using the PCR technique. Nontuberculous Mycobacteria were identified as an endosymbiont that was also more prevalent in biofilm samples, with *M. gordonae* being the most common species.

Dust and biofilms

In a study carried out in a public hospital in the city of Porto Alegre, state of Rio Grande do Sul, Carlesso[23] identified the presence of FLA samples collected from dust and biofilms in 15 different hospital environments. All the analyzed environmental samples indicated the presence of FLA, except for the ICU. A total of 35% of all collected samples were positive for FLA, and 34% of them belonged to the *Acanthamoeba* spp. genus. Among the dust samples, the kitchen was the area with the highest number of FLA isolates, while among the biofilm samples, the drinking-fountains showed the highest FLA prevalence.

Lasjerdi[24] investigated the occurrence of FLA in immunodeficiency units of hospitals in Tehran, Iran. Dust and biofilm samples from wards serving transplant, pediatric (malignancies), HIV, leukemia and oncology patients of five university hospitals were collected and examined for the presence of FLA using culture and molecular approaches. A little more than half of samples were positive for the presence of FLA, and *Acanthamoeba* genotype T4 was the most prevalent among the isolates. The presence of the T4 genotype in medical instruments, including an oxygen mask in an isolation room of a pediatric immunodeficiency clinic, should be of concern to health authorities. The *Acanthamoeba* T5, *Hartmannella vermiformis* and *Vahlkampfia avara* genotypes were also present.

Clinical samples and water

Bagheri[18] performed a study at Royal Hobart Hospital in Tasmania to evaluate the possibility of FLA colonization of the respiratory and urinary tract of intensive care patients. Patients' clinical samples and water samples were collected from the ICU, and all of them were cultured and tested using the PCR method. *Acanthamoeba* spp. was isolated from two patients' samples collected one week apart and one from a sink of an ICU patient. The first patient's sample showed marked *Acanthamoeba* spp. growth and was collected while the patient was intubated. The second sample showed moderate growth and was collected after the patient was extubated, when he was transferred to the general ward. Although the colonization of the respiratory tract of ICU patients with *Acanthamoeba* spp. may seem to be a rare event, this study showed that it may occur, and is not commonly detected because very specific methods are required for the clinical diagnosis. This fact further reinforces the role of *Acanthamoeba* spp. also as a carrier of bacterial pathogens in the airways of intensive care patients.

Disinfection treatments

Ventilation and air conditioning systems provide an effective way for airborne transmission of contaminants that may be present in the hospital environment. Microorganisms resistant to disinfection, even at small quantities, constitute a potential infection risk to individuals.[25] *Acanthamoeba* spp. are caused by their cysts and trophozoites. These structures have been found in air, soil and water sources, where treatment methods and laboratory analyses are inefficient to detect or remove them.[26] The innumerable gaps in knowledge about the presence of these parasites in the environment and the ineffectiveness of disinfection procedures are important factors for the dissemination of these pathogens.[27] Nowadays, physical and chemical methods are used to clean and disinfect water in order to inactivate cysts and oocysts. Several studies have been designed to guide sanitary professionals regarding the most effective methods.[28]

Some methods have been tested for the elimination of protozoans such as chlorine and its derivatives, Ozone, Interaction mechanisms, Ultraviolet Light, Solar Radiation and Boiling. The choice of the disinfection method for inactivation of these parasites should take into account the most appropriate cost and benefit, and not pose a risk to the population.[29]

In a recent study, researchers tested three disinfectants, of which none could completely eradicate FLA, even at higher concentrations than those recommended by the manufacturers, thereby supporting a deeper investigation of the antimicrobial spectra of commercial disinfectants under use for the maintenance and disinfection of air conditioning units.[30]

Hospital heating, ventilation and air-conditioning (HVAC) systems play an important role in filtering and circulating air, providing an adequate environment for patients and personnel. In addition to using bleach and cleaning air devices, some authors suggested that the implementation of the preventive maintenance program doubled the benefits of the research were doubled; the reliability of the HVAC equipment increased and high utility costs, which were caused by frequent breakdowns and poor utilization of machines and employees, were significantly reduced. The researchers recommend the preventive maintenance of the HVAC system in all hospitals, which should lead to good health promotion. They suggest that a well-designed preventive maintenance program is a good start for a hospital that does not have

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the resources to invest in automated cleaning systems.

Acanthamoeba spp. and air quality in hospitals

Some studies have shown that infections due to lack of air quality control in hospital settings may be associated with fungal, bacterial and protozoal contamination. The presence of antimicrobial resistant bacteria in air samples highlighted the possibility that they cause severe nosocomial infections. In Saudi Arabia, an investigation carried out at a large local hospital highlighted the presence of high amounts of fungi of the *Cladosporium* and *Penicillium* genera, being superior to what is indicated in the air quality guidelines.[33] In Brazil, a study carried out in the state of Piauí that evaluated the presence of the fungal microbiota of air conditioning devices in the ICU of public and private hospitals also indicated that air conditioners should be cleaned fortnightly.[34]

The World Health Organization has shown concern about indoor air quality, and despite the current standards, these are not always followed, leading to the difficulty in air quality maintenance in hospital settings.[35] The air quality in hospital environments is related to the adequate maintenance and cleaning of air conditioning systems, since they can become sources for the formation of microbial biofilms and trigger the process of pathogen dissemination.[36] Due to the opportunistic nature of *Acanthamoeba* spp. and its possible role as a pathogen reservoir, the monitoring of this protozoan in hospital environments becomes important and can be used as an air quality biomarker in hospital settings.

Thus, the importance of understanding the nature of the presence of *Acanthamoeba* in the internal environment, especially in air conditioning systems. Its possible role as carrier of bacteria can become a potential danger to debilitated patients.[1] As *Acanthamoeba* spp. is ubiquitous, its use as an air quality marker should be considered a biosafety measure in further studies, aiming to overcome obstacles to date insuperable regarding nosocomial infections.

CONCLUDING REMARKS

Knowledge of the indoor air quality plays an important role in preventing hospital infections and due to the opportunistic nature of *Acanthamoeba* spp. and its reservoir association with other pathogens, we can suggest their use as an important biomarker for air quality control.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Danielly Joani Bullé and Marilise Brittes Rott and Lisianne Brittes Benittez: contributed to the conception, design of the article, analysis and writing of the article;

Danielly Joani Bullé: contributed to the planning and design of the article, review and final approval of the article;

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