Research, Society and Development, v. 13, n. 10, e43131047039, 2024
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v13i10.47039

Probiotics as a therapeutic alternative in amoebiasis. Where are we?

Probióticos como alternativa terapêutica na amebíase. Onde estamos?

Probióticos como alternativa terapéutica en la amebiasis. ¿Dónde estamos?

Received: 09/24/2024 | Revised: 10/06/2024 | Accepted: 10/07/2024 | Published: 10/11/2024

Vivian Moura de Oliveira
ORCID: https://orcid.org/0000-0001-6454-630X
Universidade Federal de Minas Gerais, Brazil
E-mail: vivian-moura@live.com
Matheus Rezende Corrêa
ORCID: https://orcid.org/0009-0004-5498-1833
Universidade Federal de Minas Gerais, Brazil
E-mail: matheusrezendebh@gmail.com
Isabela Cristina Vieira Silva
ORCID: https://orcid.org/0009-0007-4126-3033
Universidade Federal de Minas Gerais, Brazil
E-mail: isabelacvsilva@gmail.com
Larissa Rezende Corrêa
ORCID: https://orcid.org/0009-0001-2010-7740
Universidade Federal de Minas Gerais, Brazil
E-mail: larissarezendec2@gmail.com
Maria Aparecida Gomes
ORCID: https://orcid.org/0000-0002-7263-5721
Universidade Federal de Minas Gerais, Brazil
E-mail: magomes@icb.ufmg.br

Abstract
Entamoeba histolytica is the cause of amoebiasis, a neglected tropical disease and a leading cause of mortality worldwide. The objective of this review is to show the potential of probiotics as a treatment strategy for amoebiasis. A systematic search was conducted using PubMed and Google Scholar with keywords "*Entamoeba histolytica*", "amoebiasis", and "probiotics." The studies on probiotics for amoebiasis treatment are limited, but some shown promising results. *Lactobacillus acidophilus* reduced *E. histolytica* cyst excretion by 79.67% in mice, with a 68.31% efficacy comparable to metronidazole. *Bifidobacterium* sp. showed 75.63% efficacy in reducing infection severity, higher than metronidazole (67.56%). *Lactobacillus salivarius* inhibited *E. histolytica* growth in vitro and provided protection in vivo. *Lactobacillus fermentum* and *L. delbrueckii* inhibited *E. histolytica* proliferation with efficacy ranging from 46.42% to 97.68%, depending on the concentration. *Saccharomyces boulardii* reduced amoebic dysentery duration by 25% and eliminated parasitic cysts in human stool samples. *Lactobacillus casei* showed a 100% recovery rate in rats infected with *E. histolytica*. These findings suggest probiotics have potential as therapeutic agents for amoebiasis, but further research is needed to confirm their effectiveness in animal models and humans.
Keywords: *Entamoeba histolytica;* Probiotics; Protozoa.

Resumo
Entamoeba histolytica é a causa da amebíase, uma doença tropical negligenciada e uma das principais causas de mortalidade mundial. O objetivo desta revisão é mostrar o potencial dos probióticos como estratégia de tratamento para amebíase. Foi realizada uma busca sistemática no PubMed e Google Scholar com as palavras-chave "*Entamoeba histolytica*", "amebíase" e "probióticos." Os estudos sobre o uso de probióticos no tratamento da amebíase são limitados, mas alguns apresentaram resultados promissores. O *Lactobacillus acidophilus* reduziu a excreção de cistos de *E. histolytica* em 79,67% em camundongos, com eficácia de 68,31%, comparável ao metronidazol. O *Bifidobacterium sp.* mostrou 75,63% de eficácia na redução da gravidade da infecção, superior ao metronidazol (67,56%). O *Lactobacillus salivarius* inibiu o crescimento de *E. histolytica* in vitro e ofereceu proteção in vivo. *Lactobacillus fermentum* e *L. delbrueckii* inibiram a proliferação de *E. histolytica* com eficácia variando de 46,42% a 97,68%, dependendo da concentração. O *Saccharomyces boulardii* reduziu a duração da disenteria amebiana em 25% e eliminou os cistos parasitários em amostras de fezes humanas. *Lactobacillus casei* apresentou uma taxa de recuperação de 100% em ratos infectados por *E. histolytica*. Esses achados sugerem que os probióticos têm potencial terapêutico contra infecções por *E. histolytica*, destacando a necessidade de mais pesquisas tanto em modelos animais quanto em humanos para validar sua aplicação no tratamento da amebíase.
Palavras-chave: *Entamoeba histoltytica*; Probióticos; Protozoáio.

Resumen

Entamoeba histolytica es la causa de la amebiasis, una enfermedad tropical desatendida y una de las principales causas de mortalidad a nivel mundial. El objetivo de esta revisión es mostrar el potencial de dos probióticos como estrategia de tratamiento para amebíase. Se realizó una búsqueda sistemática en PubMed y Google Scholar utilizando las palabras clave "*Entamoeba histolytica*", "amebiasis" y "probióticos". Los estudios sobre el uso de probióticos para tratar la amebiasis son limitados, pero algunos muestran resultados prometedores. *Lactobacillus acidophilus* redujo la excreción de quistes de *E. histolytica* en un 79,67% en ratones, con una eficacia del 68,31%, comparable al metronidazol. *Bifidobacterium sp.* mostró una eficacia del 75,63% en la reducción de la gravedad de la infección, superior al metronidazol (67,56%). *Lactobacillus salivarius* inhibió el crecimiento de *E. histolytica* in vitro y proporcionó protección in vivo. *Lactobacillus fermentum* y *L. delbrueckii* inhibieron la proliferación de *E. histolytica* con una eficacia que varía del 46,42% al 97,68%, dependiendo de la concentración utilizada. *Saccharomyces boulardii* redujo la duración de la disentería amebiana en un 25% y eliminó los quistes parasitarios en muestras de heces humanas. *Lactobacillus casei* mostró una tasa de recuperación del 100% en ratas infectadas con *E. histolytica*. Estos hallazgos sugieren que los probióticos tienen un potencial terapéutico contra las infecciones por *E. histolytica*, destacando la necesidad de más investigaciones en modelos animales y en humanos para validar su uso en el tratamiento de la amebiasis.

Palabras clave: *Entamoeba histoltytica*; Probióticos; Protozoario.

1. Introduction

The first historical account of *Entamoeba histolytica* was documented by Fedor Lösch in 1875, who observed the feces of a rancher suffering from dysentery. The term *Entamoeba histolytica* was introduced by Schaudinn in 1903, based on his observations of the parasite's notable capacity to lyse tissues. The prevalence of *E. histolytica*, the pathogen responsible for amoebiasis, is currently difficult to determine due to the emergence of *E. dispar*, which is morphologically indistinguishable from *E. histolytica* (Imperato, 1981).

Infections caused by *E. histolytica* can be asymptomatic or symptomatic. Before the recognition of *E. dispar*, it was estimated that approximately 10% of the global population was infected with *E. histolytica*, with around 10% of these individuals experiencing invasive symptomatic infections (Walsh, 1986). This suggests that at least 1% of the global population may present with symptomatic *E. histolytica* infections, leading to over 100,000 reported deaths annually. It is crucial to note that amoebiasis primarily affects individuals in developing countries, largely due to inadequate sanitation and water treatment facilities, although infections are also reported in travelers visiting endemic regions (Carrero et al., 2020).

The treatment of amoebiasis and other intestinal diseases varies according to the severity of the infection. However, the wide range of side effects, coupled with the potential for drug resistance, has led the WHO to recommend the use of probiotics and their metabolites to prevent intestinal infections caused by parasites and other microorganisms. In light of this, numerous studies have emerged to evaluate the efficacy of these probiotic compounds in treating various health conditions (Cuellar-Guevara & Menchaca-Arredondo, 2019).

Few clinical and experimental studies have investigated the effects of different probiotic strains on amoebiasis (Sarid & Ankri, 2022). Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host, primarily through the modulation of intestinal microbiota and reinforcement of immune barriers (Sanders et al., 2013). Probiotics may play a beneficial role in the prevention and treatment of parasitic infections, including amoebiasis, by promoting the recovery of intestinal microbiota and modulating the inflammatory response (Montalban-Arques et al., 2015; Sarao & Arora, 2017).

Considering the scarcity of studies relating amoebiasis and probiotics, our objective was to identify, analyze, and synthesize clinical and experimental studies evaluating the use of probiotics as therapeutic agents in infections caused by *Entamoeba histolytica*. Therefore, the central question of this review is: What is the efficacy of probiotics as therapeutic agents in the treatment of infections caused by *Entamoeba histolytica*? The analysis integrates findings on clinical outcomes related to the use of probiotics in amoebiasis, providing a comprehensive overview of the current state of research. Studies published

between 2003 and the first half of 2024 were selected through a systematic search using combinations of the keywords '*Entamoeba histolytica*,' 'amoebiasis' and 'probiotics' in the electronic databases PubMed and Google Scholar. Clinical and experimental articles that utilized probiotics as therapeutic agents and were published in English were included.

2. Methodology

Scientific Methodology is important for a research to have reproducibility in the best way possible (Pereira et al., 2018). The methodology followed a systematic (Mattos, 2015; Gomes & Caminha, 2014) process aimed at ensuring the comprehensiveness and relevance of the selected studies, as well as the standardization of data analysis.

2.1 Inclusion criteria

Only articles published in English, between 2003 and 2024, that investigated the use of probiotics as treatment in in vivo and/or in vitro experiments involving *Entamoeba histolytica* were included. The search was conducted in the electronic databases PubMed and Google Scholar, utilizing combinations of the keywords "*Entamoeba histolytica*," "amoebiasis," and "probiotics." The research followed a systematic approach, employing Boolean operators to ensure that the articles addressed the topics of interest in an integrated manner.

2.2 Exclusion criteria

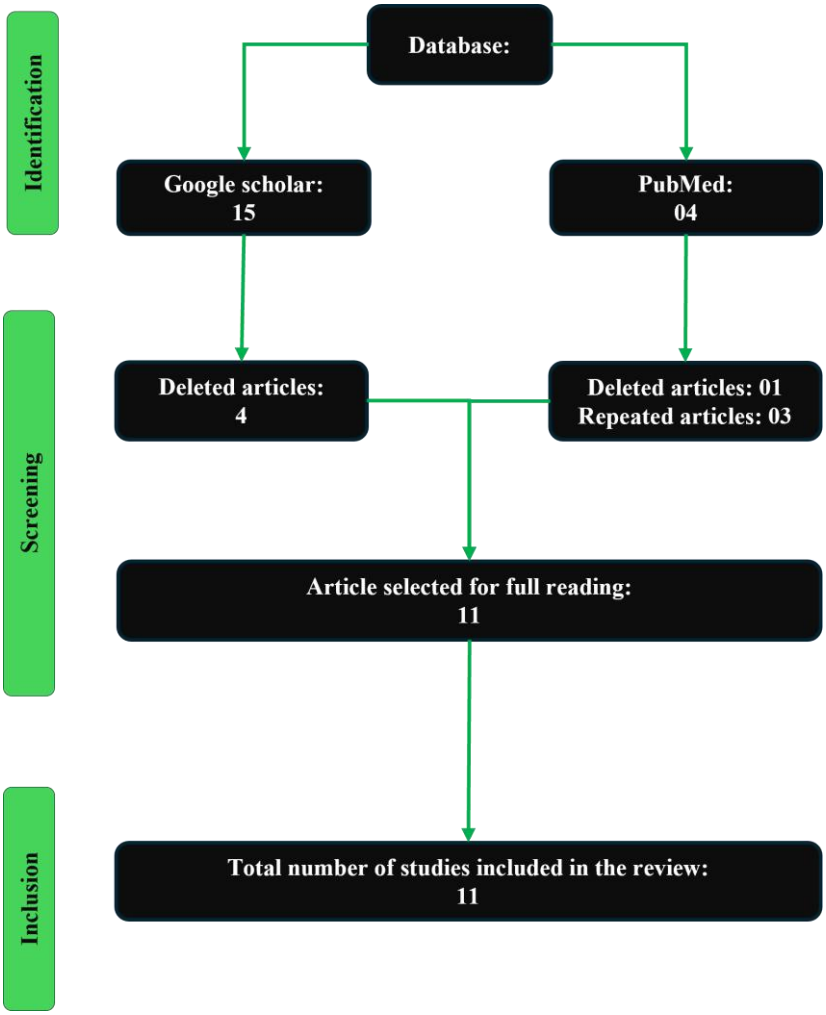
Repeated or duplicate articles in different databases, literature reviews and studies that tested probiotics in amoebas other than *Entamoeba histolytica* were excluded.

2.3 Data organization

Following the initial search, the selection of studies was conducted in multiple stages (Figure 1). First, titles and abstracts were reviewed to identify those that met the inclusion criteria. The studies that passed this preliminary screening were then read in their entirety for a more detailed evaluation. Subsequently, the studies were assessed for methodological quality and their relevance to the objectives of the review. The evaluation criteria included clarity in experimental methods, robustness of results, and appropriateness of statistical analysis.

The results were categorized based on the type of experiment, the probiotic utilized, and the efficacy of the probiotics reported in each study. The analysis included a comparison of the observed effects in the treatment of infections caused by *E. histolytica*. Additionally, the consistency of the results across different studies was evaluated, along with the potential impact of variations in the type of probiotic used.

Figure 1 – Flowchart of the Article Selection Process Steps.



Source: Prepared by the authors (2024).

3. Literature review

3.1 *E. histolytica* and Amoebiasis

Amoebas are unicellular protozoa belonging to the phylum Sarcodina, characterized by the absence of a fixed shape due to their amoeboid movement through pseudopods (Adl et al., 2019). These cytoplasmic extensions allow amoebas to move and capture food particles, such as bacteria and other microorganisms, through a process called phagocytosis. Living in various aquatic and moist environments, amoebas are highly adaptable organisms that can survive in both water bodies and wet soils. Their simple structure includes a nucleus, cytoplasm, digestive and contractile vacuoles, and they reproduce asexually, typically through binary fission (Samba-Louaka & Héchard, 2019).

The life cycle of the parasite is relatively simple, presenting itself in the forms of cysts and trophozoites. The infection process occurs via the fecal-oral route, without the need for a vector, where cysts, the resistant form of the parasite eliminated in the host's feces, are ingested through contaminated food or water. Upon reaching the terminal ileum, excystation occurs, releasing trophozoites into the host's intestine, which then migrate to the colon, continuing their life cycle (Guillén, 2023).

The epidemiology of *E. histolytica*/*E. dispar* infections is characterized by a high number of asymptomatic cases, where the parasite remains in equilibrium with the host, perpetuating disease transmission through the release of cysts in feces.

However, approximately 10% of infected individuals progress to more severe forms of the disease, including amoebic colitis, amoeboma of the colon, and fulminant colitis, all symptoms that fall under intestinal amoebiasis, caused by *E. histolytica* (Morán et al., 2023).

In certain instances, clinical manifestations may occur beyond the gastrointestinal tract, primarily through hematogenous dissemination via the portal vein. This pathway can facilitate the migration of *Entamoeba histolytica* trophozoites to the liver, where they can induce the formation of amoebic liver abscesses. Additionally, these trophozoites may reach the pulmonary system, resulting in pulmonary amoebiasis, which can similarly lead to the development of abscesses. In rarer cases, *E. histolytica* may also affect the brain (Abasszade & Bell, 2021; Uribe-Querol & Rosales, 2020).

Prevention of amoebiasis involves good hygiene practices, such as regular handwashing, treating water before consumption, and ensuring proper food preparation. Basic sanitation measures and education on hygiene are crucial for reducing disease incidence, especially in vulnerable communities. Although amoebiasis is often easily treatable with specific medications, it can be dangerous if not diagnosed and treated adequately, highlighting the importance of attention to the quality of water and food, particularly in at-risk areas.

3.2 *Entamoeba histolytica* vs intestinal microbiota

The intestinal microbiota performs its function in four different environments in the human body: metabolic, structural, protective, and neurological. In the protective environment, the mucus layer consists of mucin glycoproteins secreted by goblet cells, forming a viscous gel-like layer on the intestinal epithelium. This layer serves to prevent microbial adhesion directly to the epithelium and also functions as a lubricant, facilitating the transport of luminal contents without damaging the epithelial lining (Johansson et al., 2008).

The mucus layer is approximately 150 µm thick and comprises two distinct strata of sialomucins and sulfomucins arranged alternately. The acidic sulfomucin is located closest to the epithelium and is less susceptible to degradation by bacterial glycosidases and host proteases. Additionally, this acidic mucin plays a critical role in preventing the direct adherence of commensal microorganisms to colonic epithelial cells (Johansson et al., 2008).

When *E. histolytica* infects a healthy individual with an intact intestinal barrier, the amoebas adhere to the outer mucus layer, away from the epithelium. In this context, mucus plays a protective role by maintaining a healthy balance between the microbiome and pathogens, as both are in constant competition for binding sites within the mucus layer (Pelaseyed et al., 2014; Sperandio & Sansonetti, 2015). The adhesion of *E. histolytica* to the intestinal mucus layer is mediated by a surface lectin that exhibits high affinity for galactose (Gal) and N-acetyl-D-galactosamine (GalNAc) oligosaccharides found in mucin, the primary component of the mucus layer (Birchenough & Hansson, 2015).

Initial infection by *Entamoeba histolytica* leads to thickening of the mucosal layer, likely as a defense mechanism to prevent the pathogen from contacting the intestinal epithelium. During the infection, amoebae produce glycosidases and proteases that can degrade the mucin layer, exposing the intestinal epithelial cells (IECs) (Lidell & Hansson, 2006; Moncada & Chadee, 2005). In the absence of mucin, the amoebic lectin Gal/GalNAc binds to Gal and GalNAc residues on the surface of the exposed IECs. Progressive disease is characterized by mucin loss, flattening of IECs, and neutrophil infiltration. Furthermore, secretory molecules from *E. histolytica* disrupt tight junctions and ion transport in the intestine, leading to diarrhea. In mucin-deficient mice, for example, *E. histolytica* directly attached to IECs, resulting in increased pathology, barrier disruption, and secretory and pro-inflammatory responses (Kissoon-Singh & Chadee, 2013). Amebic lesions in the intestinal epithelium can progress to necrotic ulcers containing trophozoites, bacteria, and inflammatory cells. From these

ulcers, trophozoites may invade the tissue and enter the bloodstream, often spreading to the liver and causing amoebic liver abscesses.

4. Results

In the synthesis of results, we used narrative analysis (Whittemore & Knafl, 2005), which facilitates the integration and descriptive presentation of findings from various studies, highlighting how each contributes to the understanding of the role of probiotics in the treatment of amoebiasis. Frame I present the studies included in the sample, organized by author and year, detailing the type of probiotic used in the research, the main findings, and the type of experiment conducted, whether in vitro or in vivo.

Frame 1 - Efficacy of Probiotics in inhibiting *Entamoeba histolytica* in vivo and in vitro studies.

Study	Probiotic/Treatment	Results and effectiveness (%)	Type of experiment
Mansour-Ghanaei et al., 2003	*Saccharomyces boulardii*	100% reduction in parasitic cysts and 50% reduction in episodes of fever and abdominal pain	In humans
Dinleyici et al., 2009	*Saccharomyces boulardii*	Bloody diarrhea resolved within 5 days in group B, 76% to 24% reduction in diarrhea within 36 hours	In humans
Savay-Erdeve et al., 2009	*Saccharomyces boulardii*	Reduction of amoebic cysts: 8,8% (Group I) and 7,5% (Group II)	In humans
Khalaf, 2013	*Lactobacillus acidophilus*	79,67% (protection), 68,31% (treatment), Significant reduction in cysts excretion	In vivo
Rahi & Nashaat, 2013	*Lactobacillus casei*	80% (group 1), 100% (group 2), watery diarrhea control	In vivo
Mohamed, 2014	Bifidobacteria sp.	75,63%, complete resolution on the eighth day after inoclation	In vivo
Mohammed et al., 2015	*Lactobacillus salivarius*	More effective inhibition than metronidazole in vitro	In vitro and in vivo
Shafeek & Ruzzuki, 2016	*Lactobacillus fermentum e Lactobacillus delbrueckii*	46,42% ($1,5 \times 10^2$), 88,65% ($1,5 \times 10^4$), 97,68% ($1,5 \times 10^8$), inhibition of growth of *E. histolytica*	In vitro
Sabti et al., 2019	*Lactobacillus acidophilus*	Gradual reduction of cysts in infected mice	In vivo
Sarid et al., 2022	*Lactobacillus acidophilus*	50% reduction in trophozoite viability, effect mediated by H_2O_2	In vitro
Das et al., 2024	Bacterial isolates	Significant elimination of *E. histolytica* in mice	In vivo

Source: Prepared by the authors (2024).

5. Discussion

Experimental trials involving probiotics have emerged as a growing area of research, driven by the recognition of the beneficial effects of these live microorganisms on human health (Magistrelli et al., 2019; Maldonado & Perdigón, 2019; Mazziotta & Rotondo, 2023). Probiotics, such as *Lactobacillus* and *Bifidobacterium*, are commonly studied for their ability to enhance intestinal microbiota, strengthen the immune system, and prevent gastrointestinal diseases (Al-Tawaha & Meng, 2018; Flach et al., 2018). These trials aim to evaluate the efficacy, safety, and mechanisms of action of probiotics under various health conditions, providing scientific evidence for their therapeutic and preventive use. Experimental research on probiotics is essential for validating their clinical effects and establishing appropriate usage protocols.

There are very few studies addressing the topic amoebiasis and probiotics. However, in all the studies reviewed, probiotics have been shown to be at least feasible adjuncts in the therapy of amoebiasis. It has been demonstrated that

administering the probiotic *Lactobacillus acidophilus* for seven days prior to exposing mice to *E. histolytica* trophozoites significantly reduced cyst excretion. Additionally, infected mice treated with *L. acidophilus* showed a gradual decrease in cyst numbers starting from the first day of treatment. The protection conferred by the probiotic was 79.67%, while the efficacy of the treatment was 68.31%, compared to an efficacy of 67.56% for metronidazole (Khalaf, 2013).

In another study, *L. acidophilus* was observed to gradually reduce the number of *E. histolytica* trophozoites present in the feces of infected mice (Sabti, et al., 2019). Incubation with *L. acidophilus* for two hours resulted in a 50% reduction in the viability of *E. histolytica* trophozoites. However, this amoebicidal activity was lost in the presence of catalase, an enzyme that degrades hydrogen peroxide (H_2O_2) into water and oxygen. This finding suggests that the effect may be mediated by H_2O_2 produced by *L. acidophilus* (Sarid et al., 2022).

One more study evaluated the effect of Bifidobacteria in BALB/c mice infected with *E. histolytica*. The results indicated that the administration of *Bifidobacterium* spp. for seven days prior to infection demonstrated 75.63% efficacy in reducing cyst elimination and the severity of the infection, with complete resolution by the eighth day post-inoculation. The excretion of *E. histolytica* cysts was significantly higher in groups treated with metronidazole, which showed 67.56% efficacy but did not eradicate the infection until the tenth day (Mohamed, 2014).

Lactobacillus salivarius, a probiotic bacterium present in the gastrointestinal tract with various therapeutic properties, has also been investigated for its antiamoebic activity. The supernatant containing the bacteriocin from *L. salivarius* was tested both in vitro and in vivo against *E. histolytica*, using metronidazole as a reference treatment. The results indicated that the bacteriocin from *L. salivarius* effectively inhibited the growth of *E. histolytica* in vitro, while the supernatant provided superior protection in vivo (Mohammed, 2015).

Studies involving *Lactobacillus fermentum* and *Lactobacillus delbrueckii* have demonstrated that these probiotic bacteria are effective in inhibiting the growth of *E. histolytica*. The treatment, conducted at various concentrations of the probiotic, proved effective across all tested concentrations, achieving an inhibition rate of 97.68% at a concentration of 10^8 cells/ml after 72 hours of incubation (Shafeek & Ruzzuki, 2016).

A randomized, double-blind clinical trial was conducted involving 57 adults diagnosed with intestinal amoebiasis, characterized by acute mucous diarrhea accompanied by fever and abdominal pain. Participants were randomly assigned to two groups. Group 1 received a combination of metronidazole and iodoquinol three times daily for 10 days. Group 2 received the same regimen as Group 1, with the addition of lyophilized *Saccharomyces boulardii*, also administered three times daily for 10 days. Follow-up assessments were conducted at 2 and 4 weeks post-treatment. Results indicated that the inclusion of the probiotic significantly reduced the duration of amoebic dysentery by approximately 25% and decreased the incidence of fever and abdominal pain by nearly 50%. In terms of parasitic cysts in fecal samples, Group 1 demonstrated an 81.5% reduction, while the probiotic group achieved a complete reduction (100%) of the parasitic cyst form elimination (Mansour-Ghanaei & Shafaghi, 2003).

Another randomized clinical trial involving 53 children selected based on clinical symptoms (fever, bloody diarrhea, and abdominal pain) divided the participants into two groups. Group A received metronidazole treatment twice daily, while Group B was treated with both metronidazole and *Saccharomyces boulardii*, also administered twice daily. The results indicated that the resolution of bloody diarrhea occurred more rapidly in Group B. A similar trend was observed for the cessation of diarrhea, with a shorter duration noted in Group B compared to Group A. When both symptoms were assessed over a 48-hour period, they were significantly more prevalent in Group A. After 36 hours, only 24% of children in Group B continued to experience diarrhea, compared to 76% in Group A; additionally, 12% of children in Group B still exhibited blood in their stools, whereas this figure was 44% in Group A. Over a five-day period, all children in Group B were free of blood in

their stools and did not present with trophozoites or cysts. By the end of ten days, all children in both groups had fully recovered based on the parameters evaluated in the study (Dinleyici, 2009).

In another study, forty-five children in Group I received only oral metronidazole for 10 days, while another 40 in Group II received *Saccharomyces boulardii* in addition to the same medication. The primary outcomes investigated included the duration of acute and bloody diarrhea, the frequency and consistency of stool, the time taken for symptom resolution, as well as the tolerance and side effects associated with the treatment regimens. The results indicated that the rate of children with amoebic cysts two weeks after the cessation of treatment was 8.8% in Group I and 7.5% in Group II (Savas-Erdeve & Dallar, 2009).

A study was conducted involving 45 rats suffering from watery diarrhea. All fecal samples contained trophozoites of *Entamoeba histolytica*. The rats were divided into three groups: the first group received 2 ml of *Lactobacillus casei* extract orally, the second group received 2 ml of *L. casei* suspension, and the third group received 2 ml of normal saline as a positive control. The first group exhibited an 80% recovery rate, the second group demonstrated a 100% recovery rate, while the control group showed no recovery (Rahi & Nashaat, 2013).

Another recent study selected twenty bacterial isolates based on their ability to tolerate acid and bile salt. The selected isolate was assessed for in vivo safety using a mouse model, demonstrating significant activity in the elimination of *E. histolytica* (Das & Dam, 2024).

Despite the limited number of studies evaluating the amoebicidal activity of probiotics and their role in inhibiting amoebic proliferation, the findings highlight the therapeutic potential of probiotics as an alternative or adjunctive treatment for amoebiasis. Given the scarcity of research in this field and the promising therapeutic effects of probiotics, particularly in intestinal infections, this review encourages further investigation into the use of probiotics for amoebiasis, aiming to establish their role in the management of this condition.

6. Final Considerations

Based on the studies presented it is evident that different probiotic strains, such as *Lactobacillus acidophilus*, *Bifidobacterium*, *Lactobacillus salivarius*, *Lactobacillus fermentum*, and *Lactobacillus delbrueckii*, demonstrated significant efficacy in inhibiting and controlling infections caused by *Entamoeba histolytica*. These probiotics were shown to reduce cyst excretion, decrease the viability and proliferation of trophozoites, and in some cases, completely eliminate the infection in both in vivo and in vitro models. The effectiveness of the probiotics varied depending on the strain and the study conditions; however, in many instances, their efficacy was comparable to or exceeded that of the standard treatment with metronidazole, highlighting the therapeutic potential of probiotics as complementary or alternative agents in the treatment of amoebiasis.

These findings underscore the significance of further investigating probiotics as a promising and safe strategy for treating *E. histolytica* infections. This is particularly important due to their potential to decrease parasitic load while minimizing the common side effects associated with traditional medications. Future research should aim to elucidate the mechanisms by which probiotics exert their effects, optimize dosing regimens, and assess their efficacy in human subjects. This will contribute to the development of a broader range of therapeutic options for managing these infections.

Acknowledgments

VMO and MAG are grateful to CNPq and FAPEMIG for the research grant.

Conflict of Interest

The authors declare no conflicts of interest.

References

Abasszade, J. H., Little, R., Yeaman, F., Robertson, M., & Bell, S. (2021). Amoebic colitis: A case series of a recurring missed diagnosis. *JGH Open*, *5*(3), 404-407.

Adl, S. M., Bass, D., Lane, C. E. et al. (2019). Revisions to the classification, nomenclature, and diversity of eukaryotes. *Journal of Eukaryotic Microbiology*, *66*(1), 4-119.

Al-Tawaha, R., & Meng, C. (2018). Potential benefits of Lactobacillus plantarum as probiotic and its advantages in human health and industrial applications: A review. *Adv. Environ. Biol*, *12*, 16-27.

Birchenough, G. M., Johansson, M. E., Gustafsson, J. K., Bergström, J. H., & Hansson, G. (2015). New developments in goblet cell mucus secretion and function. *Mucosal immunology*, *8*(4), 712-719.

Carrero, J. C., Reyes-López, M., Serrano-Luna, J., Shibayama, M., Unzueta, J., León-Sicairos, N., & de la Garza, M. (2020). Intestinal amoebiasis: 160 years of its first detection and still remains as a health problem in developing countries. *International Journal of Medical Microbiology*, *310*(1), 151358.

Cuellar-Guevara, F. L., Barrón-González, M. P., & Menchaca-Arredondo, J. L. (2019). Effect of Lactobacillus postbiotics on Entamoeba histolytica trophozoites. *Revista de investigación clínica*, *71*(6), 402-407.

Das, M., Biswas, P., & Dam, S. (2024). Isolation and Potential Probiotic Characterization of Lactic Acid Bacteria from an Ethnic Fermented Beverage having Antibacterial Activity against Human Pathogen Entamoeba histolytica. *International Journal of Pharmaceutical Investigation*, *14*(2).

Dinleyici, E., Eren, M., Yargic, Z., DOĞAN, N., & Vandenplas, Y. (2009). Clinical efficacy of Saccharomyces boulardii and metronidazole compared to metronidazole alone in children with acute bloody diarrhea caused by amebiasis: a prospective, randomized, open label study. *American Journal of Tropical Medicine and Hygiene*, *80*(6).

Flach, J., Van Der Waal, M. B., Kardinaal, A. F. M., Schloesser, J., Ruijschop, R. M. A. J., & Claassen, E. (2018). Probiotic research priorities for the healthy adult population: A review on the health benefits of Lactobacillus rhamnosus GG and Bifidobacterium animalis subspecies lactis BB-12. *Cogent Food & Agriculture*, *4*(1), 1452839.

Gomes, I. S. & Caminha, I. O. (2014). Guia para estudos de revisão sistemática: uma opção metodológica para as Ciências do Movimento Humano. *Movimento*. 20 (1), 395-411.

Guillén, N. (2023). Pathogenicity and virulence of Entamoeba histolytica, the agent of amoebiasis. *Virulence*, *14*(1), 2158656.

Imperato, P. J. (1981). A historical overview of amebiasis. *Bulletin of the New York Academy of Medicine*, *57*(3), 175.

Johansson, M. E., Phillipson, M., Petersson, J., Velcich, A., Holm, L., & Hansson, G. C. (2008). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proceedings of the national academy of sciences*, *105*(39), 15064-15069.

Khalaf, K. J. (2013). Study of Lactobacillus acidophilus as atreatment and prophylaxis for Entamoeba histolytica infected mice. *World Journal of Pharmaceutical Research*, *2*(5), 1229-1237.

Kissoon-Singh, V., Moreau, F., Trusevych, E., & Chadee, K. (2013). Entamoeba histolytica exacerbates epithelial tight junction permeability and proinflammatory responses in Muc2−/− mice. *The American journal of pathology*, *182*(3), 852-865.

Lidell, M. E., Moncada, D. M., Chadee, K., & Hansson, G. C. (2006). Entamoeba histolytica cysteine proteases cleave the MUC2 mucin in its C-terminal domain and dissolve the protective colonic mucus gel. *Proceedings of the National Academy of Sciences*, *103*(24), 9298-9303.

Magistrelli, L., Amoruso, A., Mogna, L., Graziano, T., Cantello, R., Pane, M., & Comi, C. (2019). Probiotics may have beneficial effects in Parkinson's disease: in vitro evidence. *Frontiers in immunology*, *10*, 969.

Maldonado Galdeano, C., Cazorla, S. I., Lemme Dumit, J. M., Vélez, E., & Perdigón, G. (2019). Beneficial effects of probiotic consumption on the immune system. *Annals of Nutrition and Metabolism*, *74*(2), 115-124.

Mansour-Ghanaei, F., Dehbashi, N., Yazdanparast, K., & Shafaghi, A. (2003). Efficacy of Saccharomyces boulardii with antibiotics in acute amoebiasis. *World journal of gastroenterology: WJG*, *9*(8), 1832.

Mattos, P. C. (2015). Tipos de revisão de literatura. Unesp, 1-9. https://www.fca.unesp.br/Home/Biblioteca/tipos-de-evisao-de-literatura.pdf 6.1.3).

Mazziotta, C., Tognon, M., Martini, F., Torreggiani, E., & Rotondo, J. C. (2023). Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*, *12*(1), 184.

Mohamed, S. T. (2014). Use of Bifidobacterium for Treatment and Protective from Entamoeba Histolytica Infection in Mice. *International Journal of Science and Technology*, *3*(1).

Mohammed, S., Khalaf, K., & Sulaiman, N. (2015). Lactobacillus salivarius bacteriocin and supernatant activity against Entamoeba histolytica in vitro and in vivo. *Br J Biol Health Med Sci Res*, *3*, 19-28.

Moncada, D., Keller, K., & Chadee, K. (2005). Entamoeba histolytica-secreted products degrade colonic mucin oligosaccharides. *Infection and immunity*, *73*(6), 3790-3793.

Montalban-Arques, A., De Schryver, P., Bossier, P., Gorkiewicz, G., Mulero, V., Gatlin III, D. M., & Galindo-Villegas, J. (2015). Selective manipulation of the gut microbiota improves immune status in vertebrates. *Frontiers in Immunology*, *6*, 512.

Morán, P., Serrano-Vázquez, A., Rojas-Velázquez, L., González, E., Pérez-Juárez, H., Hernández, E. G., ... & Ximénez, C. (2023). Amoebiasis: Advances in diagnosis, treatment, immunology features and the interaction with the intestinal ecosystem. *International Journal of Molecular Sciences*, *24*(14), 11755.

Pelaseyed, T., Bergström, J. H., Gustafsson, J. K., Ermund, A., Birchenough, G. M., Schütte, A., ... & Hansson, G. C. (2014). The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Immunological reviews*, *260*(1), 8-20.

Pereira A. S. et al. (2018). Metodologia da pesquisa científica. [free e-book]. Santa Maria/RS. Ed. UAB/NTE/UFSM. 6.1.2).

Rahi, A. A., & Nashaat, Z. (2013). Leaky cells and the use of Lactobacillus casei in the treatment of laboratory animals infected with amoebic dysentery. *Euphrates Journal of Agriculture Science*, *5*(1), 15-22.

Sabti, Z. S., Abbas, S. K., & Mahmood, N. N. (2019). Investigation the Role of Lactobacillus ssp. on Entamoeba histolytica Pathogenesis. *Indian Journal of Public Health Research & Development*, *10*(10).

Samba-Louaka, A., Delafont, V., Rodier, M. H., Cateau, E., & Héchard, Y. (2019). Free-living amoebae and squatters in the wild: ecological and molecular features. *FEMS microbiology reviews*, *43*(4), 415-434.

Sanders, M. E., Guarner, F., Guerrant, R., Holt, P. R., Quigley, E. M., Sartor, R. B., ... & Mayer, E. A. (2013). An update on the use and investigation of probiotics in health and disease. *Gut*, *62*(5), 787-796.

Sarao, L. K., & Arora, M. (2017). Probiotics, prebiotics, and microencapsulation: A review. *Critical reviews in food science and nutrition*, *57*(2), 344-371.

Sarid, L., Zanditenas, E., Ye, J., Trebicz-Geffen, M., & Ankri, S. (2022). Insights into the mechanisms of Lactobacillus acidophilus activity against Entamoeba histolytica by using thiol redox proteomics. *Antioxidants*, *11*(5), 814.

Savaş-Erdeve, S., Gökay, S., & Dallar, Y. (2009). Efficacy and safety of Saccharomyces boulardii in amebiasis-associated diarrhea in children. *The Turkish Journal of Pediatrics*, *51*(3), 220-224.

Shafeek, Z., & Ruzzuki, N. (2016). The effect of different concentrations of the run and stuck bacteria (Lactobacillus fermentum, Lactobacillus delbrueckii) in trophozoite growth of Entamoeba histolytica in vitro. *Int J Curr Microbiol Appl Sci*, *5*, 924-30.

Sperandio, B., Fischer, N., & Sansonetti, P. J. (2015). Mucosal physical and chemical innate barriers: Lessons from microbial evasion strategies. In *Seminars in immunology*, Vol. 27, No. 2, pp. 111-118.

Uribe-Querol, E., & Rosales, C. (2020). Immune response to the enteric parasite Entamoeba histolytica. *Physiology*, *35*(4), 244-260.

Walsh, J. A. (1986). Problems in recognition and diagnosis of amebiasis: estimation of the global magnitude of morbidity and mortality. *Reviews of infectious diseases*, *8*(2), 228-238.

Whittemore, R., & Knafl, K. (2005). The integrative review: updated methodology. *Journal of advanced nursing*, *52*(5), 546-553.