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Blastocystis sp.: A Hidden Player in Gut Health and Disease

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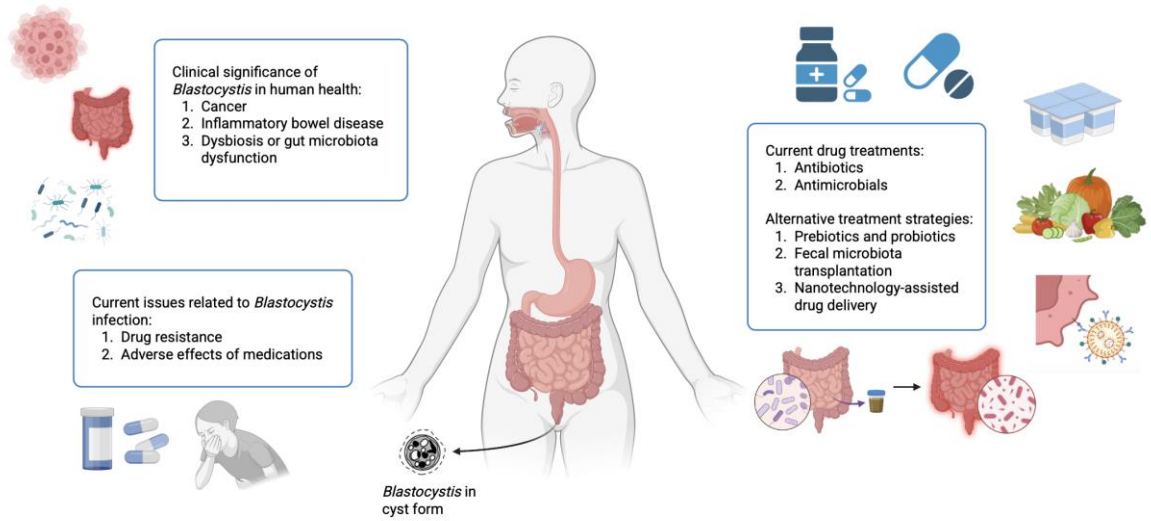
Abstract: *Blastocystis* sp. (*Blastocystis*) is a prevalent and diverse gastrointestinal parasite, found in up to 60% of humans and animals worldwide, with particularly high rates in immunocompromised individuals and those in close contact with animals. Its extensive genetic diversity presents a major challenge to diagnostic and treatment standardization, making subtype characterization essential for identifying transmission pathways and

assessing infection risks. The clinical significance of *Blastocystis* is increasingly evident, as it is implicated in a range of gastrointestinal conditions, including irritable bowel syndrome, inflammatory bowel disease, and chronic diarrhea. Its interactions with the host's immune system and gut microbiome add layers of complexity, shaping the parasite's impact on health and disease in unpredictable ways. This review examines the multifaceted role of *Blastocystis* in human health, exploring its association with gastrointestinal disorders, cancer, and dysbiosis, while also evaluating current treatment options and emerging therapeutic strategies. A deeper understanding of these interactions could unlock more effective, targeted interventions for managing *Blastocystis* infections and the conditions they may influence.



Graphical abstract. Clinical significance and management of *Blastocystis* infection: *Blastocystis* is associated with cancer, inflammatory bowel disease, and gut microbiota dysbiosis. Current challenges include drug resistance and adverse effects of medications. Standard treatments involve antibiotics and antimicrobials, while alternative approaches such as probiotics, fecal microbiota transplantation, and nanotechnology-assisted drug delivery are being explored.

Keywords: *Blastocystis*; parasites; pathogenesis; subtypes; treatment; SDG 3 Good health and well-being

1. Introduction

Blastocystis sp. (*Blastocystis*) is a common intestinal parasite affecting over one billion people worldwide[1] . The prevalence of *Blastocystis* varies globally and it is one of the most common single-celled eukaryotes found in human fecal samples[2]. Extensive studies have documented *Blastocystis* prevalence in China, Nigeria, Malaysia, Lebanon, and Brazil, indicating its global impact[3–7]. Its ability to infect diverse groups, from rural populations to refugees, showcases its adaptability[8]. Once considered harmless, recent

studies suggest a link between *Blastocystis* infections and gastrointestinal disorders such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and chronic diarrhea[2], renewing interest in its biology, pathogenicity, and epidemiology[9].

The clinical significance of *Blastocystis* is debated, influenced by subtype diversity, host immune response, and gut microbiome interactions[10]. Its colonization is associated with increased gut microbiota diversity, potentially affecting the gut ecosystem[11]. Studies on *Blastocystis*-host immune system interactions reveal insights into how the parasite influences immune responses, potentially leading to chronic inflammation and tissue damage[12]. Immunocompromised individuals, such as those with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) or cancer, are more susceptible to *Blastocystis*[9]. Understanding these interactions is key to developing targeted therapies.

Blastocystis exhibits considerable genetic diversity with at least 17 subtypes, complicating diagnosis and treatment[13]. Genetic diversity complicates standardized diagnostic methods and treatment protocols[14,15]. Human-pathogenic subtypes pose public health risks, emphasizing the need to understand subtype distribution[16]. Molecular tools, such as real-time polymerase chain reaction (PCR) assays, enable subtype identification, aiding in epidemiological investigations and treatment decisions[17,18]. Advances in molecular techniques provide insights into genetic diversity and evolutionary relationships among *Blastocystis* subtypes[19,20].

Research on *Blastocystis* and gut microbiota dysbiosis links it to chronic diseases such as IBD and IBS[21]. *Blastocystis* impacts gut microbiota composition, contributing to gastrointestinal disorders[22]. Conversely, other subtypes are linked to gut microbiota dysbiosis and negative health effects, emphasizing the need for subtype-specific research[23]. Molecular biology techniques, including next-generation sequencing and metagenomics, offer new insights into the epidemiology and genetic diversity of *Blastocystis*[24]. These technologies help identify novel subtypes and their distribution, providing valuable information on the parasite's evolutionary history and transmission dynamics[25]. Studies suggest certain *Blastocystis* subtypes, such as ST1, may ameliorate colitis and promote beneficial microbiota and immune outcomes[26].

The variability in clinical presentation, from asymptomatic carriage to severe distress, challenges diagnosis and management[27]. Understanding the interplay between *Blastocystis*, the gut microbiota, and host immunity advances knowledge of its pathogenic mechanisms and aids in developing targeted therapeutic interventions. Effective management strategies require a comprehensive understanding of the epidemiology, risk factors, and genetic diversity of *Blastocystis*[28]. Studies on the antimicrobial susceptibility of *Blastocystis* isolates

highlight the need for effective treatments[29]. Subtype characterization aids in identifying transmission sources and routes[30] and understanding transmission dynamics and infection risks[31]. This review provides a comprehensive overview of the impacts of *Blastocystis* infection in various diseases such as cancer, IBD, and dysbiosis. The current drug treatments for *Blastocystis* infection and other alternative treatment strategies are also discussed in detail.

2. Understanding *Blastocystis* Subtypes

The prevalence of *Blastocystis* remains unknown in many parts of the world. However, identifying specific subtypes can help uncover their genetic diversity and associated disease risks. *Blastocystis* is genetically diverse and is classified into 28 subtypes (ST) based on polymorphic regions across small subunit ribosomal RNA (SSU rDNA) or 18S rRNA gene analysis. The various subtypes of *Blastocystis* identified were ST1-ST17, ST21, ST23-ST29, and ST30-ST32[32]. The different subtypes of *Blastocystis* are indistinguishable under the microscope due to their similar morphological forms. Among the 28 subtypes, at least 22 subtypes of *Blastocystis* were described. In both humans and animals, ten *Blastocystis* subtypes (ST1 to ST9 and ST12) are frequently identified. These subtypes can be transmitted through infected individuals, contaminated food, water, or environmental sources. On the other hand, there are another 12 subtypes of *Blastocystis* which are isolated exclusively from animals. These subtypes can be transmitted to humans who work closely with animals. For example, ST5 in pigs, ST10 in goats, sheep, and cattle; as well as ST6 and ST7 in chickens[33].

The subtypes of *Blastocystis* identified in the Asia region were ST 1-14, while ST 18-22 were identified in Asia as novel subtypes. In terms of genetic diversity, *Blastocystis* is also being classified at the intra-subtype level. ST 1-4 are the most frequently isolated subtypes around the world, and they comprise up to 90% of the *Blastocystis* genotype. Meanwhile, ST 5-9 are rarely detected. Various *Blastocystis* subtypes are associated with distinct clinical symptoms upon infection and require specific identification methods[34]. Identifying the various subtypes of *Blastocystis* is crucial for controlling and preventing infection by targeting potential sources, such as contaminated water, soil, food, and zoonotic transmission risks. In contaminated water and soil, ST1 and ST3 are commonly isolated, indicating shared *Blastocystis* transmission risks between humans and animals through these sources[35].

3. Transmission Pathways of *Blastocystis* into the Human Host

Blastocystis enters the human body in its cyst form via the fecal-oral route when feces-contaminated food and water are orally ingested[32]. The risk of infection increases in areas with polluted water supply, as well as contamination in food preparation and handling. The trophozoite form of *Blastocystis* undergoes asexual multiplication with various pleiomorphic stages in the small intestine. This developmental cycle of *Blastocystis* eventually gives rise to non-invasive colonization in the human intestinal tract[36]. Clinical symptoms of *Blastocystis* infection include abdominal pain, bloating, and flatulence[37]. According to a study conducted in Brazil, food can also be contaminated indirectly. The ingestion of raw or undercooked vegetables such as lettuce (*Lactuca sativa L.*) and coriander (*Coriandrum sativum L.*) has exposed consumers to *Blastocystis* cysts. The cysts on vegetables remained infective and viable for days. Such contamination usually occurs due to poor sanitary conditions, untreated manure, application of biosolids as fertilizers, presence of *Blastocystis*-infected animals, or contamination of irrigation water and soil. The foodborne infection of *Blastocystis* raises severe health problems, especially in developing countries. In developed countries, foodborne transmission of *Blastocystis* cysts in humans is mostly via large-scale food production and distribution of contaminated raw ingredients in containers[38].

The interactions between humans and animals in terms of poultry, livestock, domestic pets, as well as wildlife conservation have greatly contributed to the zoonotic transmission of *Blastocystis* into human hosts[39]. Individuals working in close contact with domestic or wild animals are very susceptible to infections caused by the diverse subtypes of *Blastocystis*[40]. Zoonotic transmission of *Blastocystis* can also occur mechanically through environmental vectors such as crawling insects, flying insects, and mollusks, or through exposure to infected feces from animals like dogs and cows. These animal feces can contaminate the environment, increasing the distribution and transmission range of *Blastocystis* from animals to humans[41].

A study in a chicken abattoir found that mishandling can lead to the direct transmission of *Blastocystis* subtypes 6 and 7 from chickens to humans; these subtypes have also been observed in pigs in several provinces in China. Additionally, subtype 8 has been recorded in transmissions between zookeepers and primates. Subtypes 1, 7, 10, and 23 are also implicated in animal-to-human transmission. The presence of *Blastocystis* in soil also contributes to its entry into the human body, largely due to the extensive use of animal feces as organic fertilizer. Wild animals may be exposed to food contaminated with *Blastocystis* cysts in the soil, indirectly facilitating animal-to-human transmission[42]. Common *Blastocystis* subtypes found in soil include ST1, ST3, ST7, ST23, and ST26. Soil should be

thoroughly tested for *Blastocystis* presence, especially for agricultural purposes. This suggests a shared transmission cycle among humans, animals, water, and soil, with ST1 and ST3 being the most transmissible[43].

There are no clear transmission patterns of *Blastocystis* between humans, animals, the environment, or through food and water. These independent routes contribute to the parasite's prevalence in humans[44]. Communities in undeveloped or rural areas are more prone to *Blastocystis* infection due to contaminated food, polluted water, and low hygiene awareness. This highlights the importance of community structure and environmental factors in determining *Blastocystis* transmission, whether waterborne, foodborne, zoonotic, or human-to-human[45]. The transmission among humans and from animals is not as frequent as from the environment or food due to the high exposure to water and soil, and the increase in awareness of personal hygiene in handling food[46]. *Blastocystis* can also be transmitted into the human body via fecal microbiota transplantation without asymptomatic gastrointestinal complications. Due to the treatment of *Clostridioides difficile* infections, patients are treated with fecal microbiota transplantation (FMT) by using feces provided by healthy donors. Apart from feces-contaminated food or water, feces can also be colonized by *Blastocystis*, which contributes to pathogenicity and is transferred to the patient. To prevent gastrointestinal symptomatology caused by fecal transmission of *Blastocystis*, a molecular screening test can be carried out to prevent *Blastocystis* infection[47]. In summary, there are several routes of entry of *Blastocystis* into the human body, which include contaminated water or food, zoonotic transmission, environmental transmission via water or soil, as well as fecal microbiota transplantation treatment.

4. *Blastocystis* Pathogenicity

Blastocystis was documented in 1870 as a non-pathogenic parasite[48]. However, the pathogenicity of *Blastocystis* remains controversial due to the varied conclusions drawn from numerous epidemiological and experimental animal studies[49]. They cause infection in individuals regardless of the symptoms, indicating their occurrence in both symptomatic and asymptomatic patients[9]. The literature demonstrated that symptomatic infection is associated with gastrointestinal disorders, including IBS, colorectal cancer, abdominal pain, ulcerative colitis[50–53], and extra-gastrointestinal symptoms, including chronic spontaneous urticarial, weight loss, cutaneous lesions, and joint pain[50,53,54].

A significant challenge in studying the pathogenesis is the absence of suitable animal models[54]. Previously, laboratory mice infected with *Blastocystis* exhibited several symptoms, such as lethargy, weight loss, and demonstrated increased vulnerability to

infections as they age[55]. However, other studies carried out on BALB/c mice showed immunity against blastocystosis[9,55,56]. In studies involving rats and chickens, variable infection outcomes were observed. Notably, both species showed immunity against *Blastocystis* subtype 3, while subtype 7 only infects chickens[56,57]. In rats, *Blastocystis* infection triggered intense inflammatory reactions, indicating disrupted tight junctions between intestinal epithelial cells, which led to impaired barrier function and increased permeability[57]. Mortality rates in rats vary by *Blastocystis* subtype, with subtype 1 causing higher mortality than subtypes 3 and 4. Subtype 4 is a weaker genotype but upregulates proinflammatory cytokines (IL-12, interferon-γ, and TNF-α) as a protective response[58]. Humans host diverse intestinal microbiota that influence *Blastocystis* pathogenicity, potentially transforming the protozoan from harmless to harmful, either as commensals or parasites[59].

In vitro cell culture systems have been utilized to study the mechanisms of *Blastocystis* pathogenesis. Inoculation with *Blastocystis* subtype 1 has been shown to modulate the immune response in mammalian cell cultures by inducing the production of granulocyte-macrophage colony-stimulating factor and IL-8[60]. Similarly, subtype-4 stimulated IL-8 formation by secreting cysteine proteases in a nuclear factor κB-dependent manner[9,60]. This cysteine protease, like those found in other protozoans such as *Acanthamoeba*[61] and *Entamoeba*, also induces epithelial cell dysfunction through mechanisms involving the ROCK-dependent disruption. This process resulted in the disruption of cytoskeletal F-actin and modifications to tight junctions, contributing to cellular restructuring[62]. The protozoa interacted with epithelial cells and induced apoptosis in host cells through a contact-independent mechanism[9,60]. These proteases were also capable of cleaving secretory immunoglobulin, i.e., IgA, present at the mucosal surface[9,63]. Enhanced secretion of this enzyme elevated the pathogenic potential of *Blastocystis* via IgA degradation. The protist attached to epithelial cells using various carbohydrates (N-acetyl-β-D-glucosamine, α-D-glucosyl, and α-D-mannosyl) present on its surface, allowing it to colonize and multiply in the host (Figure 1)[64].

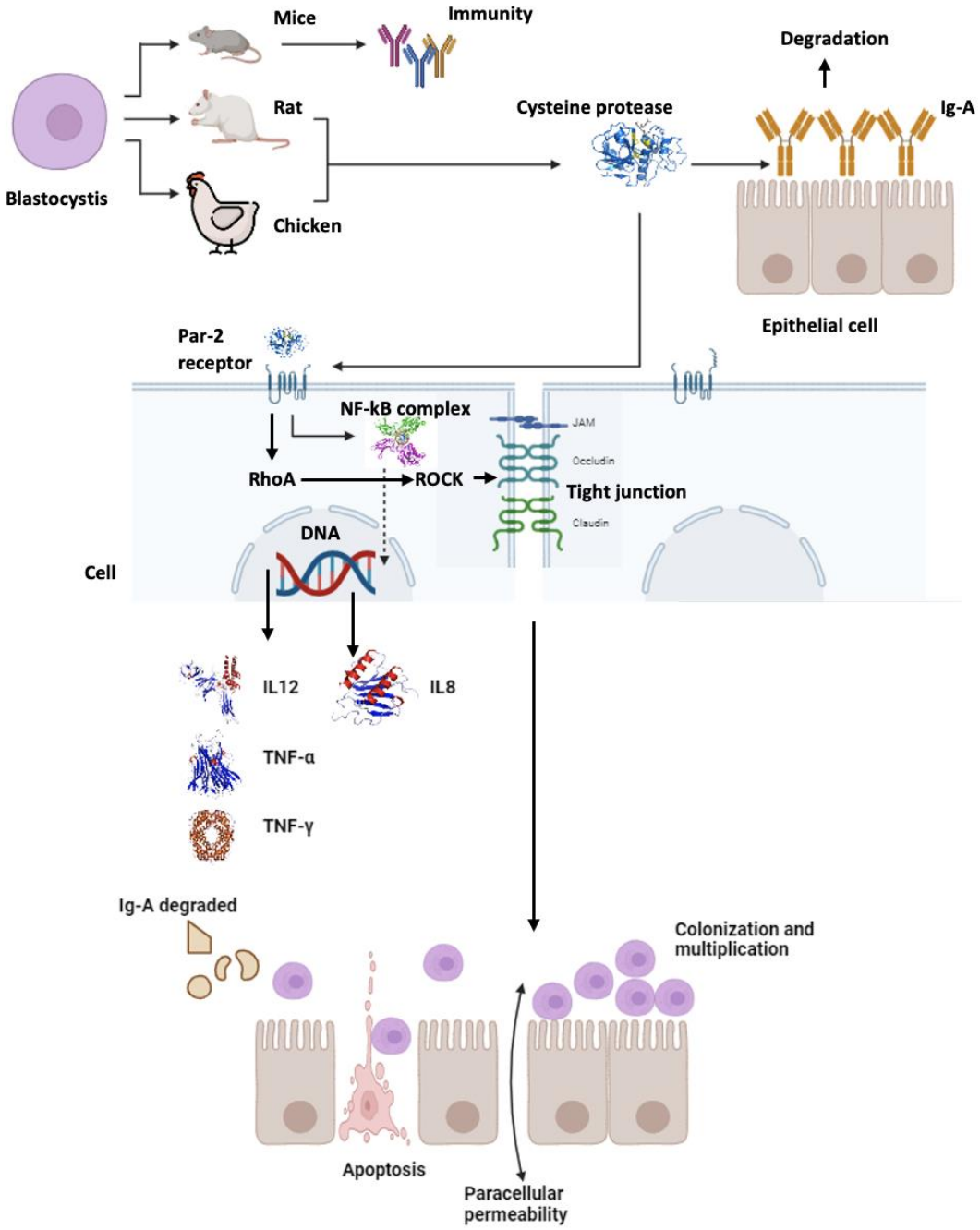


Figure 1. A proposed cellular model for the pathogenesis of *Blastocystis* at the molecular level. Mice, rats, and chickens can be used as a model for *Blastocystis sp*. However, mice have shown an immunity to pathogens. Variability in outcomes is observed when chickens and rats are used as models. The pathogen secretes cysteine protease, which degrades IgA within epithelial cells. This enzyme subsequently impairs epithelial cell function through a ROCK-dependent mechanism. Finally resulted in apoptosis in epithelial cells through a contact-dependent manner. The enzyme also secretes IL 12, TNF-α, and TNF-γ, in a nuclear factor κB-dependent manner.

Studies have indicated that differences in the pathogenicity of *Blastocystis* infection were associated with genetic diversity. Subtype 1 was reported as pathogenic, while subtype 2 was considered non-pathogenic[65]. This study suggests that variations in pathogenicity

across different geographical regions may indicate that parasitic isolates exhibit distinct mechanisms of pathogenesis in each area[66]. The phenotypes also have a greater impact on the pathogenicity of the parasite. Pathogenic symptomatic subtypes are present in amoeboid form and grow much faster as compared to their non-pathogenic counterparts[67]. The pathogen releases hyaluronidase enzymes, which degrade extracellular matrix proteins, creating space and facilitating their invasion[68]. Other phenotypic variations among different pathogenic subtypes have been demonstrated through molecular characterization, protein profiling, and isoenzyme patterns[69]. The antigen isolated from symptomatic *Blastocystis* exhibits higher pathogenicity by impairing immune response as compared to asymptomatic *Blastocystis*[70].

The disease is more common in mentally retarded (MR) patients and patients with an immune system deficiency[71]. Similarly, young mice are more susceptible to the infection as compared to older mice, which develop more resistance than younger ones[64]. The infection of *Blastocystis* also caused skin allergy symptoms, which were due to the secretion of IgE in an immune response to the pathogen's antigens[72]. These observations suggested a potential role for *Blastocystis* in the onset of clinical symptoms, thereby supporting its involvement in pathogenesis.

5. Impacts of Blastocystis Infection on Human Health

Infectious agents have long been considered significant risk factors in the development of various cancers, with emerging evidence highlighting the potential role of parasitic infections in cancer pathogenesis. Among these, *Blastocystis* has garnered increasing attention due to its potential virulence role and its association with several types of cancers, as indicated by multiple studies[73]. Research has shown that *Blastocystis* infection can lead to a broad spectrum of gastrointestinal symptoms, such as abdominal pain, loose stools, diarrhea, constipation, nausea, bloating, flatulence, cramps, and fatigue. These symptoms often overlap with those seen in IBS, which suggests a potential link between *Blastocystis* and gut dysfunction[74]. Moreover, it is demonstrated that *Blastocystis* infection could lead to an imbalance of microbiota in the gut (dysbiosis) by altering the microbiome of gut bacteria[75].

5.1. Cancer

A recent study by Kumarasamy *et al*. in Malaysia found that *Blastocystis* antigen isolated from an unknown subtype could facilitate the proliferation of colon cancer cells[76]. The current study compared the effects of solubilized antigens isolated from five different

subtypes of *Blastocystis* on colon cancer cells (HCT116). Significant cell proliferation was observed when exposed to 1.0 μg/ml solubilized antigen isolated from *Blastocystis* subtype 3. The study also showed upregulation of Th2 cytokines, especially transforming growth factor beta, in subtype 3-treated cancer cells. Of interest, *Blastocystis* subtype 3 also caused a notable increase in cathepsin B expression, which may contribute to the exacerbation of colon cancer by weakening the immune response.

A previous study found that the solubilized antigen of *Blastocystis hominis* caused severe cytopathic and immunological effects in human colorectal cancer (CRC) cells, potentially increasing their proliferative, invasive, and metastatic properties[77]. A 2019 study in Tehran, Iran, showed a high prevalence of *Blastocystis* in cancer patients, including those with CRC (28.2%)[78]. Another study demonstrated that *Blastocystis* plays a significant role in enhancing Azoxymethane (AOM)-induced carcinogenesis damage to the intestinal epithelium and oxidative damage, suggesting a possible association between subtype 1 of *Blastocystis hominis* and CRC. A study in China by Zhang et al. showed that *Blastocystis* subtype 1 was more prevalent than subtype 3 in CRC patients with diarrhea, with 66.7% of patients infected with ST1[79]. A study in Saudi Arabia found that *Blastocystis* was present in 29.7% of CRC patients, particularly subtype 1 (54.5%), suggesting a possible carcinogenic effect[80]. A study in Uzbekistan[81] showed higher prevalence of *Blastocystis* in CRC patients (80%) compared to controls, with a significant association between the presence of *Blastocystis* and both non-metastatic (T1–4N0M0) and metastatic (T1–4N1–2M0–1) CRC patients. The frequency of *Blastocystis* was notably higher in metastatic CRC patients, with more than twice as many T1–4N1–2M0–1 patients infected compared to non-metastatic patients. These findings suggest a potential link between *Blastocystis* and carcinogenesis. A study in Malaysia found that 46.7% of CRC patients tested positive for *Blastocystis* during intermediate chemotherapy cycles[82]. However, no patients had *Blastocystis* infections during the later chemotherapy cycles, suggesting that the patients' antioxidant regulatory systems may have reacted to the chemotherapy, boosting their immune system and subsequently combating the *Blastocystis* infections. In Iraq, a study found that CRC patients co-infected with *Blastocystis* had associated pathological gene mutations[83]. A study by Sulżyc-Bielicka et al. showed a higher prevalence of *Blastocystis* in CRC patients (12.15%) compared to controls (2.42%), particularly subtype 3[84]. The odds of infection were five times higher in CRC patients, and *Blastocystis* infection rates increased with tumor stage, with stage 3 CRC being a significant predictor for positive infection. Based on the results, four subtypes (ST1, 2, 3, and 7) of *Blastocystis* were identified in the study individuals[85].

Kumarasamy *et al*. found that colon cancer cells exposed to *Blastocystis* antigen showed significantly higher levels of IL-6 and IL-8, indicating that these cytokines may be involved in the cellular immune response to *Blastocystis*. This response could involve the release of inflammatory cytokines and reactive oxygen species, contributing to carcinogenesis. Furthermore, the study also revealed dysregulation of IFN-γ and p53 expression, especially in subtype 3-treated HCT116 cells, suggesting that *Blastocystis* infection might reduce apoptosis in colon cancer cells. Since p53 is a tumor suppressor gene, its dysregulation is linked to cancer development[76]. Chan et al. found that exposure to antigens from both symptomatic and asymptomatic *Blastocystis* isolates increased cell proliferation in the colorectal carcinoma cell line HCT116. However, HCT116 cells showed a significantly higher proliferation when exposed to symptomatic *Blastocystis* antigens, even at low concentrations (0.005 μg/ml), compared to asymptomatic antigens, which required higher concentrations (5 μg/ml) for similar effects. The study also observed a significant upregulation of both Th1 (IFN-γ and TNF-α) and Th2 (IL-6, IL-8, and TGF-β) cytokines in response to symptomatic *Blastocystis* antigens. Notably, Th2 cytokines were more highly upregulated than Th1 cytokines, suggesting that symptomatic *Blastocystis* might induce a Th2-dominated immune response, potentially weakening the cellular immune response and promoting tumor cell growth[86].

Ahmed et al. found two protein bands (230 and 32 KDa) present in 42.9% of *Blastocystis* CRC isolates, which were absent in non-CRC isolates. However, there was no significant difference in the protease activity among CRC, non-CRC symptomatic, and asymptomatic patients[87]. Chandramathi et al. demonstrated that *Blastocystis hominis* antigens could promote the growth of HCT116 cells by downregulating IFN-γ and upregulating IL-6 and NF-κB gene expressions. These findings suggest that *Blastocystis* antigens might weaken the immune response in colon cancer cells while promoting their growth [88]. Kumarasamy et al. found that co-administration of *Blastocystis* led to a 1.6-fold increase in colonic crypts in rats treated with the carcinogen, AOM. The study also revealed the presence of adenomas, major dysplasia, and hyperplastic aberrant crypts in rats co-infected with *Blastocystis* and AOM[89]. Ali et al. identified *Blastocystis* in 52% of CRC and 42% of non-cancer individuals, respectively. The study found significant risk factors for *Blastocystis*-infected CRC patients, including vomiting with flatulence and previous surgery with stage 3 CRC[85] .

Mahmoudvand et al. found a significant difference in *Blastocystis hominis* prevalence between CRC patients (23.9%) and healthy individuals (9%). Factors like agricultural activity and consumption of unwashed fruit and vegetables were significantly related to the

prevalence of *Blastocystis hominis* infection[90]. Mülayim et al. reported a 14.4% prevalence of *Blastocystis* in a Turkish population, with ST3 (40%), ST2 (33%), and ST1 (20%), showing no association with cancer patient demographics or symptoms[91]. Labania *et al*. found significantly higher *Blastocystis* prevalence in CRC patients, mainly with ST3 (44.4%) and ST2 (33.3%)[92]. Öner *et al*. reported 61.5% *Blastocystis* prevalence in colon cancer patients, with ST4 (55.8%) being the most common subtype, followed by ST1 (25.6%) and ST3 (18.6%)[93]. The impacts of *Blastocystis* infection on cancer were summarized in Table 1.

Table 1. Roles of *Blastocystis* infection in cancer.

Human Disease	**Subtype of *Blastocystis***	**Mechanism of Causing the Disease**	**References**
Cancer	ST1, ST2, ST3, ST4, ST5	• Upregulation of Th2 cytokines especially transforming growth factor beta • Upregulation of cathepsin B • Upregulation of IL-6 and IL-8 • Dysregulation of IFN-γ and p53 expression	[76]
	ST1	• Enhancing AOM-induced carcinogenesis damage to the intestinal epithelium and oxidative damage	[78]
	N/A	• Increase cell proliferation of cancer cells • Upregulation of nuclear factor kappa light chain enhancer of activated B cells (NF-κB)	[86]
	Blastocystis	• Increase in the number of colonic crypts • Occurrence of adenoma • Presence of hyperplastic aberrant crypts	[89]

Collectively, the evidence suggests a possible association between *Blastocystis* infection, particularly subtypes 1 and 3, and colorectal cancer progression, potentially through mechanisms involving cytokine dysregulation, impaired immune responses, and enhanced oxidative stress. While several studies report higher *Blastocystis* prevalence among CRC patients and show that *Blastocystis* antigens can induce cancer cell proliferation, the current body of research is largely correlative, lacking mechanistic clarity and longitudinal data. Moreover, inconsistencies in subtype distribution and variation in study design, geographic location, and patient health status complicate cross-study comparisons. Many studies are limited by small sample sizes, lack of proper controls, and heterogeneity in *Blastocystis* detection methods. It remains unclear whether *Blastocystis* acts as a causative agent in carcinogenesis or merely thrives in the altered gut environment of cancer patients.

Future research should focus on controlled experimental models, subtype-specific pathogenicity, and the host's immune modulation to determine causality and to identify whether *Blastocystis* could serve as a potential biomarker or therapeutic target in CRC.

5.2. Inflammatory Bowel Disease (IBD)

Hussain et al. reported significantly elevated IgG-specific antibody levels against *Blastocystis hominis* in patients with irritable bowel syndrome (IBS) compared with healthy individuals, with IgG2 being the predominant subclass. This suggests a sustained immune response to *Blastocystis* infection, which may similarly occur in IBD due to chronic antigenic stimulation[94]. Puthia et al. showed that lysates of *Blastocystis* (ST4) induced significant IL-8 production over time and contained proteases capable of cleaving human-secreted IgA, thereby impairing mucosal immune defense. In the context of IBD, where mucosal immunity is already compromised, such immune evasion could exacerbate gut inflammation[95]. Supporting this, Wu et al. demonstrated that infection with *Blastocystis hominis* in BALB/c mice significantly increased proinflammatory cytokines IL-17 and IL-23, which are crucial in the differentiation of pathogenic Th17 cells, a key driver of inflammation in IBD[96].

Moreover, oxidative stress, a known contributor to IBD pathogenesis, was implicated by Chandramathi *et al*., who showed that certain *Blastocystis* subtypes induced high levels of reactive oxygen species during infection[88]. Kumarasamy *et al*. further confirmed elevated oxidative stress markers such as advanced oxidative protein products (AOPP) and hydrogen peroxide (H_2O_2) in *Blastocystis*-infected rats, along with increased lipid hydroperoxides, indicating membrane damage. These changes were associated with mucosal disruption and increased crypt formation in the colon, suggesting that *Blastocystis* may trigger oxidative injury and epithelial damage, a hallmark of IBD. Collectively, these findings highlight the potential role of *Blastocystis* infection in disrupting gut homeostasis, promoting inflammation, and contributing to the pathogenesis or exacerbation of IBD[89]. The impacts of *Blastocystis* infection in IBD were summarized in Table 2.

The compiled evidence indicates a plausible link between *Blastocystis* infection and the pathogenesis or exacerbation of IBD, primarily through mechanisms involving chronic immune activation, mucosal immune evasion, and oxidative stress. Several studies implicate *Blastocystis* subtypes, particularly ST4, in promoting proinflammatory cytokine responses (e.g., IL-8, IL-17, IL-23) and oxidative injury, which are key features of IBD pathology. However, the current body of work remains largely associative and based on preclinical models, with limited direct evidence from well-controlled human studies. A major limitation lies in the difficulty of disentangling cause from effect, as it is unclear whether *Blastocystis*

contributes to disease onset or preferentially colonizes already inflamed or immunocompromised intestines. Additionally, subtype-specific effects are not consistently explored, and variation in host immune status and gut microbiota further confounds interpretations. Future research should aim to clarify the causal role of *Blastocystis* using longitudinal human cohorts, explore subtype-specific immunopathogenicity, and investigate interactions with host genetics and microbiota to better understand its contribution to IBD progression.

Table 2. Impacts of *Blastocystis* infection in inflammatory bowel disease.

Human Disease	**Subtype of *Blastocystis***	**Mechanism of Causing the Disease**	**References**
Inflammatory bowel disease	*Blastocystis hominis*	Persistent antigenic exposure	[94]
	ST4	Persistent antigenic exposure	[95]
	Blastocystis hominis	Persistent antigenic exposure	[96]
	Blastocystis hominis	Oxidation stress	[88]
	Blastocystis	Oxidation stress	[89]

5.3. Dysbiosis or Gut Microbiota Dysfunction

Nourrisson *et al*. found signs of gut dysbiosis in *Blastocystis*-infected patients, primarily involving subtype ST4. *Blastocystis* carriers had over twice the levels of Saccharomycetaceae compared to non-carriers, while *Clavispora* abundance was significantly lower. Fungal composition also shifted, with increased Dipodascaceae and reduced Aspergillaceae in patients[97]. Vega *et al*. reported a significant link between *Blastocystis* and *Clostridium difficile* infection, suggesting *Blastocystis* may adapt to dysbiosis caused by oxidative stress[98]. Yason et al. conducted an *in vivo* mouse study showing that *Blastocystis* infection alters gut microbiota. Infection with subtypes ST7-B or ST7-H significantly reduced *Bifidobacterium* by day 3, while *Lactobacillus* levels decreased only in ST7-H-infected mice on days 1 and 3 post-infection. Conversely, *Escherichia coli* increased significantly in ST7-B-infected mice on days 1 and 2 post-infection. These findings suggest that *Blastocystis* may negatively impact beneficial gut microbiota[99]. Defaye *et al*. found that *Blastocystis* ST4 infection in rats led to significant changes in gut microbiota by day 31, despite no initial differences at day 0. Infected rats showed enriched bacterial diversity, increased *Proteobacteria* and *Tenericutes*, and reduced *Clostridium*, *Pseudomonas*, and *Rhodoplanes*, with higher levels of *Anaerovorax*, *Oscillospira*, and *Parabacteroides*. Additionally, short-chain fatty acids (SCFAs) like acetate and propionate

were significantly reduced, which may compromise gut health. These findings suggest *Blastocystis* can indirectly induce dysbiosis and affect gut microbial balance[100].

Nieves-Ramírez et al. reported that *Blastocystis* ST3 colonization significantly altered gut bacterial diversity, increasing species such as *Prevotella copri*, *Prevotella stercorea*, *Ruminoccoccus bromii*, *Alistipes putredinis*, *Bacteroides* species, *Bifidobacterium longum*, and *Oscillospira* species[101]. *Ruminococcus* species, including *Ruminococcus gnavus*, have been implicated in inflammatory gut diseases, suggesting potential relevance to *Blastocystis*-associated dysbiosis[102]. Functional analysis showed elevated production of SCFA, indicating fermentation pattern changes linked to dysbiosis[101]. Stensvold et al. found higher alpha diversity in *Blastocystis* carriers, with enriched *Firmicutes* and *Bacteroidetes*, while *Proteobacteria* dominated in non-carriers. Although no beta diversity difference was observed, specific bacterial genera were enriched differently, suggesting that *Blastocystis* selectively influences the gut microbiome and contributes to dysbiosis[103]. Castañeda *et al*. found that *Blastocystis*-colonized school children in Colombia had higher gut microbial richness, especially in *Firmicutes*, while *Bacteroidetes* was more abundant in non-colonized individuals. Though not statistically significant, colonized individuals showed increased diversity based on richness and Shannon indices[104]. Muñoz-Yañez *et al*. reported *Blastocystis* prevalence in both healthy (47%) and metabolically ill (65.5%) individuals, with multiple subtypes identified. Both groups showed a low *Firmicutes*/*Bacteroidetes* (F/B) ratio, a marker of dysbiosis, suggesting *Blastocystis* may be associated with altered gut microbial balance in diverse populations[105].

A study by Even *et al*. found a high prevalence of *Blastocystis* (75.4%) in the Cameroonian population, mainly subtypes ST3, ST1, and ST2. *Blastocystis*-positive individuals had higher abundances of beneficial gut bacteria, including *Ruminococcaceae*, *Butyrivibrio*, *Christensenellaceae*, *Elusimicrobium*, *Coprococcus*, *Eubacterium ruminantium*, and *Eubacterium xylanophilum*, and those colonized by multiple subtypes showed greater microbial diversity[106]. Similarly, Nieto-Clavijo *et al*. reported that healthy individuals had higher bacterial richness and diversity than those with spondyloarthritis. Among the latter, *Blastocystis* colonization was associated with increased *Pseudomonadota* and *Succinivibrionaceae*, while healthy individuals had more *Lactobacillus* species, suggesting Blastocystis may influence gut microbiome composition and contribute to dysbiosis[107]. Kodio et al. found that *Blastocystis* colonization in Malian children was associated with significantly higher gut microbiota richness and diversity compared to non-colonized children, as shown by multiple diversity indices. The permutational multivariate analysis of variance (PERMANOVA) revealed distinct differences in bacterial community

composition, with higher levels of *Firmicutes*, *Elusimicrobia*, *Lentisphaerae*, and *Euryarchaeota* in colonized children, while *Actinobacteria*, *Proteobacteria*, and *Deinococcus–Thermus* were more abundant in non-colonized children. These findings suggest that *Blastocystis* colonization is linked to increased intestinal bacterial diversity[108].

A case-control study by Behboud et al. reported that among the Iranian population, *Blastocystis* subtypes ST1(40%), ST2 (30%), and ST3 (30%) were found in asymptomatic individuals. The study showed that *Blastocystis*, *Bifidobacterium*, *Peptostreptococcus productus*, *Lactobacillus/Enterococcus*, and *Escherichia coli* were significantly upregulated, while *Bacteroides fragilis* and *Enterococcus* were significantly downregulated in *Blastocystis*-positive individuals. These findings indicate a selective influence of *Blastocysti*s on gut microbiota composition, potentially contributing to dysbiosis[109]. The impacts of *Blastocystis* infection on dysbiosis or gut microbiota dysfunction were summarized in Table 3.

The evidence surrounding *Blastocystis* and gut dysbiosis reveals a complex and often contradictory picture. Some studies associate specific subtypes (particularly ST3, ST4, and ST7) with negative shifts in gut microbial balance, including reductions in beneficial bacteria (e.g., *Bifidobacterium*, *Lactobacillus*) and short-chain fatty acids, as well as increases in opportunistic or proinflammatory taxa such as *Escherichia coli* and *Ruminococcus gnavus*. Conversely, other studies suggest that *Blastocystis* colonization, especially in asymptomatic individuals, may correlate with increased microbial richness and diversity, which are generally markers of gut health. These conflicting findings may be due to differences in study populations (healthy vs. diseased), geographical settings, detection methods, or host factors such as diet, genetics, and immunity. Furthermore, the role of specific subtypes in modulating microbial ecology remains poorly understood and inconsistently reported. Current research lacks longitudinal and mechanistic studies that can clarify whether *Blastocystis* drives dysbiosis or is simply a bystander that thrives in already altered gut environments. Future investigations should focus on subtype-specific effects using standardized models and consider host–microbe–parasite interactions to delineate the causal links between *Blastocystis* and gut microbiota dysfunction.

Table 3. Impacts of *Blastocystis* infection in dysbiosis or gut microbiota dysfunction.

Human Disease	**Subtype of *Blastocystis***	**Mechanism of Causing the Disease**	**References**
	ST1, ST2, ST3, ST4, ST5, ST7	Selective influence on gut microbiota	[97]

Dysbiosis or Gut Microbiota Dysfunction	*Blastocystis*	Oxidative stress	[98]
	ST7	• Selective influence on gut microbiota • Decrease of the beneficial bacteria *Lactobacillus* and *Bifidobacterium*	[99]
	ST4	• Selective influence on gut microbiota • ↑serine protease activity	[100]
	ST3	• Selective influence on gut microbiota • ↑ SCFA	[101]
	Blastocystis	Selective influence on gut microbiota	[103]
	Blastocystis	Selective influence on gut microbiota	[104]
	ST1, ST2, ST3, ST4, ST5, ST7	Selective influence on gut microbiota	[105]
	ST1, ST2, ST3, ST4	Selective influence on gut microbiota	[106]
	Blastocystis	Changes in antioxidant capacities and intestinal inflammation	[107]
	Blastocystis	Selective influence on gut microbiota	[108]
	ST1, ST2, ST3	Selective influence on gut microbiota	[109]

6. Current Drug Treatments and Issues of *Blastocystis*

The true pathogenicity of *Blastocystis* and the necessity of treatment remain highly debated, rendering the clinical management of this infection an intricate subject matter. Clinically significant *Blastocystis* cases are assumed to arise primarily due to the host's immune suppression, potential interactions with the intestinal microbiota, *Blastocystis* subtype, and its protease activity[110]. An effective drug for treating *Blastocystis* should have the ability to reach high concentrations in the colonic lumen, have a short transit time in the small intestine, and not be deactivated by the intestinal flora[111]. When a patient experiences symptoms like diarrhea, abdominal pain, or hives (urticaria) and no other cause is detected, *Blastocystis* may be considered responsible, and the patient must be treated to get rid of the parasite[112].

6.1. Clinical Antibiotics and Antimicrobials for Treating Blastocystis Infection

Several antimicrobial agents have been used to treat *Blastocystis* infections, such as tinidazole, nitazoxanide, emetine, trimethoprim-sulfamethoxazole (TMP-SMX), secnidazole, metronidazole, ketoconazole, paramomycin, iodoquinol, and the probiotic *Saccharomyces boulardii*[113]. Despite the wide range of options, treatment efficacy varies greatly, and concerns about antimicrobial resistance and treatment failure have been raised[114]. The most commonly prescribed antibiotic with the widest range of treatment response is metronidazole. It is prescribed in different dosage regimens (0.75 to 1.5

gm/day for ten days), given alone or in conjunction with other medications like cotrimoxazole or paramomycin[115]. Different dosage regimens of metronidazole are prescribed for 10 days, including 250 to 750 mg 3 times/day, or 1.5 g/day[115]. Although this medication is thought to be a first line of treatment, there is a wide range in treatment response, with reports ranging from 0% to 100%[111]. A 2023 double-blind, placebo-controlled, randomized trial found that metronidazole was less efficient than a placebo to treat *Blastocystis*-related gastrointestinal complaints regardless of the *Blastocystis* subtype or co-infection with other protozoa[116]. Another placebo-controlled randomized trial found that there were no significant improvements in the placebo (53% (8/15)) and metronidazole (50% (8/16)) treated groups[117].

Another antibiotic, TMP-SMX, has demonstrated a positive impact on patients' clinical symptoms and treatment success for *Blastocystis* infections. This fixed-dose combination medication is recommended as an alternate therapy, especially if metronidazole treatment is ineffective in treating symptoms. However, it is unclear if the TMP-SMX directly affects the parasite or destroys the intestinal flora that are necessary for *Blastocystis* survival[118]. Studies on subtype-dependent variation in susceptibility showed that *Blastocystis* was more susceptible to a 1:2 TMP-SMX ratio compared to a 1:5[119]. It was found that there is no subtype-dependent difference in susceptibility at this ratio, and 95–100% of cases were successfully eradicated. Patients who received TMP-SMX therapy showed 100% recovery, according to a study by Ok *et al*.[120]. In contrast, a different research found no benefit of TMP-SMX over placebo for treating children with *Blastocystis*-related recurring abdominal discomfort[121]. Furthermore, it is believed that TMP/SMX does not permanently eradicate *Blastocystis*; rather, it merely reduces the proliferation of the parasite[122]. Similar findings were also reported by Moghaddam et al., in which only 22% (4/20) of patients treated with TMP-SMX achieved complete eradication of the parasite[123].

The broad-spectrum aminoglycoside antibiotic paramomycin proved effective in treating cutaneous symptoms related to *Blastocystis*, especially urticaria[124]. When compared to metronidazole, it has shown better efficacy in particular cases[125]. Treatment with paramomycin showed an elimination rate of 77%, which was much higher than metronidazole therapy (38%)[126]. However, it was also found that in patients with treatment failure, metronidazole was used. Additionally, investigations revealed that paramomycin had no inhibitory effect on the parasite, and it may work by exhibiting its bactericidal activity to eradicate the beneficial bacteria that are necessary for *Blastocystis* survival[127].

Another broad-spectrum antiparasitic agent, Nitazoxanide, has been shown to exhibit significant anti-*Blastocystis* activity[128]. *In vitro* studies have revealed that subtype 7 has been reported to be substantially more susceptible to nitazoxanide than subtype 4[129]. Like paramomycin, nitazoxanide may also be effective in treating *Blastocystis* when metronidazole treatment fails[130]. In a randomized placebo-controlled clinical trial, administration of Nitazoxanide 500 mg 2 times/day for three days showed 86% symptomatic relief[131]. A triple antibiotic regimen including furazolidone 0.9 g, nitazoxanide 3 g, and secnidazole 3.6 g administered by rectal enema over two days showed that 79% of patients had successfully eradicated *Blastocystis* six weeks after treatment[132].

Despite the wide range of antimicrobial agents trialed against *Blastocystis*, no consensus has been reached on a standardized treatment due to the highly variable efficacy observed across studies. This variability may stem from differences in *Blastocystis* subtypes, host immune response, co-infections, or methodological inconsistencies such as diagnostic criteria and follow-up duration. For instance, while metronidazole remains the most commonly prescribed agent, its efficacy ranges from 0% to 100%, raising concerns about resistance, subtype-specific responses, or transient symptom relief rather than true eradication. Similarly, TMP-SMX shows conflicting outcomes, suggesting potential non-parasite-related mechanisms of action. Paramomycin and nitazoxanide appear more promising in certain cases, yet evidence remains limited and inconclusive. The lack of large, well-controlled, subtype-stratified clinical trials represents a significant gap in the field. Future studies should focus on subtype-specific drug susceptibility, host microbiota interactions, and standardized outcome measures to guide rational therapy development and personalized treatment strategies.

6.2. Drug Resistance and Associated Molecular Mechanisms

The potential causes of inconsistent response to *Blastocystis* therapy may involve antibiotic resistance or diversity between the parasite's strains[133]. It has also been proposed that the resistant subtypes may be present in the unresponsive patients. According to a study, subtype 3 of the *Blastocystis* species is the most common, and there are intrasubtype variations in the morphotype of subtype 3 in connection with their pathogenic potential[134,135]. Additionally, two further studies that investigated the prevalence and genetic analysis of *Blastocystis* isolates in two distinct geographic areas—Kermanshah, Iran, and West Ismailia, Egypt—corroborated these results and concluded that ST3 is the most common subtype[134,135].

Antibiotic therapy has reportedly been shown to increase the parasite population. Numerous granular forms are formed by the parasite cells, which subsequently give rise to viable vacuolar forms. When circumstances become unfavorable, the parasites may encyst and be expelled in significant quantities through the stool[136]. It's possible that some antibiotic-resistant *Blastocystis* vacuolar forms would persist. Higher amoebic and resistant vacuolar forms of *Blastocystis* demonstrate enhanced pathogenic potentials with higher cysteine protease activity[137]. Additionally, it has been proven clinically that as an asymptomatic condition progresses into a symptomatic state, the vacuolar to amoeboid form transition occurs. Therefore, it may be beneficial to search for amoeboid forms before initiating antiprotozoal treatment[134,135]. Figure 2 demonstrates the pathogenic consequence that occurs due to antibiotic resistance of *Blastocystis*.

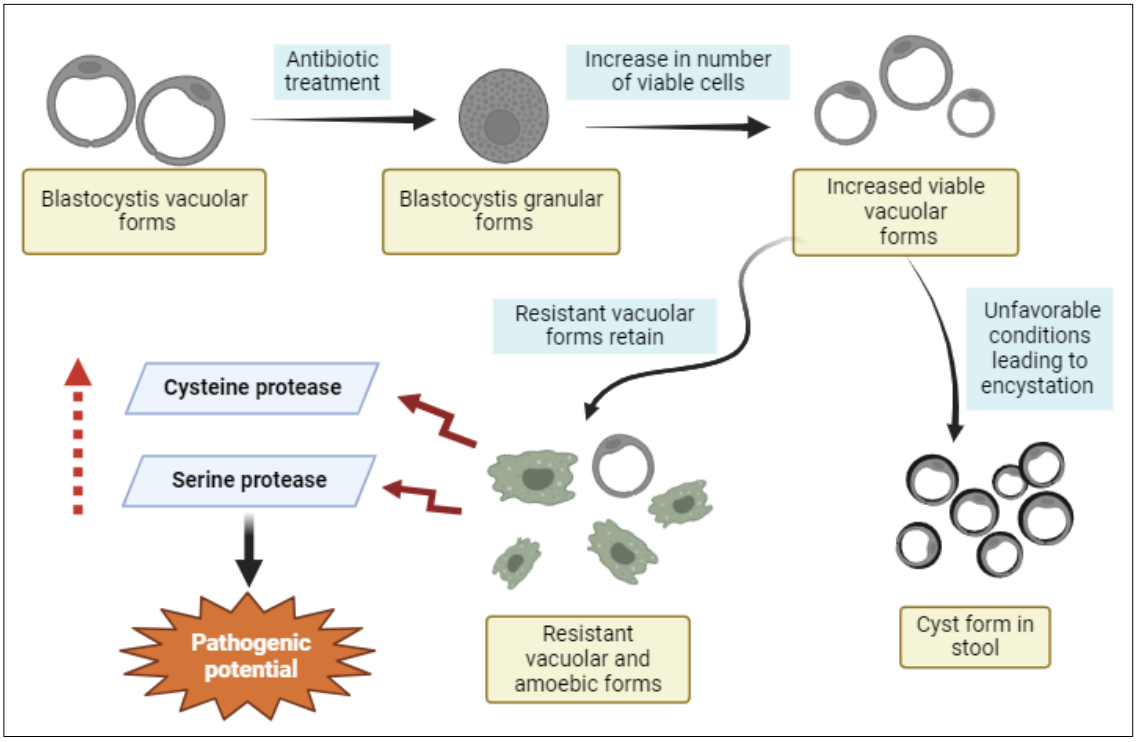


Figure 2. Resistant forms of *Blastocystis* due to antibiotic treatment.

A placebo-controlled trial showed that while metronidazole (1.5 g/day for 10 days) initially improved diarrhea and cleared *Blastocystis* in 1 month, many patients relapsed within 6 months, indicating potential resistance[138]. A study found that *Blastocystis* isolates from schizophrenia patients had higher metronidazole resistance, broader cell size ranges, and slower growth compared to non-schizophrenic individuals, who exhibited higher protease activity[137]. Resistance to both metronidazole and emetine, and cross-resistance to tinidazole in subtypes 4 and 7, suggest the presence of multidrug resistance and possibly unknown resistance mechanisms[139–141].

Placebo-controlled studies suggest that symptom recovery and complete clearance of the causative agent indicate treatment effectiveness. However, metronidazole only resolves microbiology in 80% of individuals, possibly due to its effect on the parasite's reproductive potential, causing it to become granular and release reproductive granules. This may result in increased parasite numbers in microscopy and cultures post-treatment[136,142]. Additionally, *Blastocystis* cysts are drug-resistant and genetically diverse. In axenic cultures, metronidazole induces apoptosis-like traits and promotes programmed cell death. Apoptotic bodies are stored in the central vacuole of *Blastocystis* before being released. Metronidazole also transforms into its active form in subtype 7 via reduction of ferredoxins by mitochondrion-like organelles[143,144]. Moreover, interactions in the gastrointestinal tract likely play a role in either the persistence or elimination of *Blastocystis*, with recent studies highlighting its relationship with gut microbiota and its impact on gut homeostasis[141,145,146].

In summary, the inconsistent therapeutic outcomes observed in *Blastocystis* infections are likely driven by a combination of subtype-specific variability, morphological transformations, and emerging antimicrobial resistance. Subtype 3 appears to be the most prevalent and potentially pathogenic, with morphotype diversity possibly contributing to treatment failure. The formation of drug-resistant vacuolar and amoeboid forms—along with the parasite's capacity for encystation and intracellular survival—further complicates eradication efforts. Moreover, resistance to multiple antiprotozoal drugs such as metronidazole, emetine, and tinidazole, coupled with evidence of apoptosis-like mechanisms and gut microbiota interactions, points to complex and multifactorial resistance mechanisms. These findings highlight the urgent need for improved diagnostics to detect resistant forms, tailored therapeutic strategies based on subtype identification, and further investigation into host-parasite-microbiome interactions that influence treatment efficacy and clinical outcomes.

6.3. Adverse Effects of Clinical Drug Medications against Blastocystis Infection

Many of the drugs used in the treatment of *Blastocystis* infection demonstrated significant side effects, as shown by various studies[147]. The possible side effects of metronidazole are pancreatitis and neutropenia, along with nausea, headache, and a metallic taste[148]. Additionally, alcohol usage must be avoided when this drug is administered because it may cause disulfiram-like reaction[149]. As a United States Food and Drug Administration (FDA) Pregnancy Category B drug, it should be used cautiously in the second and third trimesters of pregnancy and is contraindicated in the first[150]. On the other hand, in a research study, mice and bacteria have been reported to show mutagenic and tumorigenic activity, but

no such harmful observations were seen in over a thousand women during the 1st trimester administering this drug[151]. According to the World Health Organization (WHO), metronidazole must be avoided in lactating women as the medication accumulates in breast milk, providing an unpleasant taste that may interfere with breastfeeding[152]. Further, cerebellar ataxia and concurrent peripheral neuropathy have been linked to central nervous system adverse effects with merely 1.2 g of metronidazole and cumulative dosages exceeding 40 g[153].

TMP-SMX may serve as a better therapeutic option than metronidazole because it has fewer adverse effects. It is a medication that falls under pregnancy category C and is generally considered safe for use by pregnant women[154]. Due to the risk of kernicterus and hyperbilirubinemia in infants, TMP-SMX should not be administered to pregnant women in the near term[155]. Adverse effects related to embryocidal and teratogenic effects of TMP-SMX have been noted in animal models; however, suitable and well-controlled trials involving pregnant women have not been conducted[156]. Like metronidazole, TMP-SMX also accumulates in breast milk; however, following the newborn period, this medication is usually safe for feeding healthy, full-term infants. On the other hand, mothers of breastfeeding children who are premature, ill, jaundiced, or who lack glucose-6-phosphate dehydrogenase should generally refrain from using TMP-SMX[150].

Nitazoxanide falls under the pregnancy category B drug and is also excreted in breast milk. However, there is limited information available on the use of nitazoxanide in pregnancy, and it is unclear whether there is any risk to the developing fetus[150,157]. Children 11 years of age or under should not take nitazoxanide tablets since one tablet provides a higher dose than what is advised for pediatric dosing. According to a study, treatment with nitazoxanide showed good tolerance and did not produce any adverse effects in children[158]. Limited application of emetine is observed due to its severe side effects. Emetine is often given intramuscularly or subcutaneously because taking it orally often causes nausea and vomiting. The main cardiac toxicities are dyspnea, tachycardia, and irregularities in the electrocardiogram (ECG)[159]. Further, a combined therapy of nitazoxanide, furazolidone, and secnidazole resulted in no serious adverse event; however, urine discoloration was observed in the treated patients, which disappeared after six months[132]. The drugs prescribed in the treatment of *Blastocystis* infection and their associated adverse effects are summarized in Table 4.

Table 4. Drugs prescribed in the treatment of *Blastocystis* infection and their associated adverse effects.

Drug	Prescribed/recommended dose	Adverse effect	References
Metronidazole	Adults: 250 to 750 mg 3 times/day for 10 days, or 1500 mg/day for 7 days Pediatrics: 15 mg/kg 2 times/day for 10 days	Pancreatitis, neutropenia, nausea, headache, a metallic taste. cerebellar ataxia, peripheral neuropathy	[115]
TMP-SMX	Adults: TMP 320 mg, SMX 1600 mg (1:5 ratio)/day for 7 days Pediatrics: TMP 6 mg/kg/day for 7 days	Kernicterus and hyperbilirubinemia in infants, nausea, vomiting, skin rashes	[150]
Paromomycin	Adults: 500 mg 3 times/day for 7 days Pediatrics: 25 mg/kg 3 times/day for 10 days	Nausea, diarrhea, abdominal pain	[126]
Nitazoxanide	Adults: 500 mg 2 times/day for 3 days Pediatrics: 100-200 mg 2 times/day for 3 days	Nausea, headache, vomiting, yellowish urine, abdominal pain	[160]
Tinidazole	Adults: 2000 mg/day for 5 days Pediatrics: 50 mg/kg/day for 5 days	Nausea, vomiting, abdominal discomfort, anorexia, metallic taste	[160]
Ketoconazole	200 mg/day for 14 days	Hepatotoxicity, QT prolongation leading to irregular heartbeats, somnolence, headache	[161]
Iodoquinol	650 mg 3 times/day for 10-20 days	Pruritus ani, nausea, vomiting, abdominal discomfort, diarrhoea	[150]

While several antimicrobial drugs demonstrate varying levels of efficacy against *Blastocystis*, their clinical use is constrained by adverse effects that differ in severity, frequency, and population-specific risk. Metronidazole remains widely prescribed but presents significant neurological, gastrointestinal, and lactation-related concerns, especially with prolonged use. Alternatives like TMP-SMX and nitazoxanide offer better tolerability in some populations, yet safety data, particularly in pregnant and lactating women and pediatric groups, remain insufficient or conflicting. The scarcity of large-scale, controlled human studies on drug safety profiles underscores a major gap in the clinical management of *Blastocystis*. Moving forward, treatment decisions should weigh efficacy against the risk of adverse outcomes, particularly in vulnerable populations, and emphasize the urgent need for safer, more targeted therapies supported by robust clinical evidence.

7. Alternative Treatment Strategies

Blastocystis infections have long been difficult to treat, as conventional antibiotics frequently fail to address the intricate pathophysiology of this mysterious protozoan.

Blastocystis' biology and epidemiology have been mostly understood; however, the use of conventional antibiotics has been criticized for not being able to adequately treat the infection's complex nature. Some alternative therapy approaches shall be examined below, which have attracted more and more attention lately. These novel strategies include the use of natural compounds with antiparasitic or gut-modulating qualities, the use of probiotics and prebiotics to support a healthy gut microbiome, and the potential future use of fecal microbiota transplantation (FMT). Furthermore, the use of medication delivery methods aided by nanotechnology has the potential to improve the effectiveness and safety of these cutting-edge tactics. To ensure that these new methods can be successfully incorporated into the treatment of *Blastocystis* infections, it is imperative to stress the necessity of well-planned clinical trials to fully assess the safety and efficacy of these treatments.

7.1. Prebiotics and Probiotics to Promote a Healthy Gut Microbiome

Prebiotics and probiotics have been suggested as alternative therapy approaches for *Blastocystis* infections, as they can support a healthy gut microbiome[162]. The gut microbiome is essential for sustaining general health, and changes in its makeup have been connected to several illnesses. Common gastrointestinal protist *Blastocystis* is increasingly thought to be a commensal member of the gut microbiome; its presence is frequently linked to notable variations in the gut microbiome as well as enhanced bacterial diversity[141]. Prebiotics are non-digestible fibers that provide nutrients to good bacteria in the stomach. They can aid in the development of healthy microorganisms in the gut. This may result in a more beneficial gut microbiome, perhaps mitigating the deleterious impacts of *Blastocystis* colonization. The possible significance of probiotics, which are live microorganisms that provide health advantages when given in sufficient doses, in controlling *Blastocystis* infections has also been examined[163,164].

Probiotics coordinate complex processes that impact gut flora and enhance host health. By outcompeting harmful microorganisms for resources and habitation sites, they regulate the diversity and makeup of the gut microbiota and preserve a state of harmonious equilibrium. Probiotics interact with T cells and dendritic cells in the gut-associated lymphoid tissue to activate and modify immunological responses, which further boost immunity. This sets off the release of cytokines, which increase anti-inflammatory qualities and inhibit inflammatory factors, so enhancing immunity in general[165–167]. Probiotics also strengthen the gut epithelial barrier by decreasing permeability, which increases its defenses against pathogens and dangerous substances. They also generate SCFAs, which are beneficial to health and have anti-inflammatory properties[168]. Probiotics also prevent infections and

promote good health by blocking the growth and activity of harmful bacteria and viruses in the digestive system. Probiotics are defined as "live microorganisms with well-defined characteristics" that have a beneficial effect on immunological responses, the digestive system, and general health. Probiotics enhance gut health and balance the ratio of good to bad bacteria in the gastrointestinal tract, providing a comprehensive approach to optimizing health when combined with a balanced diet and active lifestyle[169–171]. Probiotics control stomach acidity, outcompete harmful bacteria for adhesion sites and nutrients, and make the environment unfriendly to pathogenic microorganisms. Probiotics promote anti-inflammatory responses by producing antimicrobial substances, inducing mucus production, and modulating the immune system. They also function as prebiotics, promoting the growth of good bacteria, and they control gastrointestinal motility, which influences the distribution of bacteria. These changes are influenced by treatment duration, individual responses, and strain-specific effects. Probiotic therapy tailored to the specific needs of patients suffering from gut dysbiosis can improve gut health, immunity, and overall well-being by modifying the composition of gut microbiota. The gut epithelium, which is home to many immune cells, and gut-associated lymphoid tissue (GALT) are the two main areas they affect[172,173].

Frequent prebiotic ingestion promotes variety and stability in the gut microbiota. Prebiotics, including inulin and fructo-oligosaccharides (FOS), are noteworthy because they specifically promote the growth of *Bifidobacteria* and *Lactobacilli*, which are known to be beneficial bacteria with a range of health-promoting qualities. This complex interaction highlights how important prebiotics are in creating a robust and harmonious gut environment. Prebiotics support the growth of good gut flora and can have effects on the immune system. By encouraging the production of IgA antibodies in the colon, prebiotics support mucosal immunity. IgA is necessary because it binds to toxins and pathogens to stop them from harming the gut mucosa[174]. One important mediator of these effects is the interaction of the gut microbiota with the human immune system and the GALT[175]. *Blastocystis* has been linked to a decrease in *Bacteroides* and an increase in *Firmicutes* and *Clostridiales*, two types of gut bacteria. Prebiotics could mitigate the possible adverse effects of *Blastocystis* by fostering the growth of these advantageous microorganisms[176]. *Blastocystis* has been connected to changes in the makeup of gut bacteria, including, in certain situations, a decline in good bacteria like *Bifidobacterium* and *Lactobacillus*. By encouraging the growth of these advantageous bacteria, prebiotics can aid in the restoration of a balanced gut microbiota[177]. *Blastocystis* has been linked to bacterial processes in the metabolism of aromatic amino acids, as well as pyrimidine and one-carbon metabolism mediated by folate. These metabolic processes, which are crucial for preserving a balanced gut flora, can be supported by prebiotics[176]. *Blastocystis* has been connected to changes in gut bacterial composition and

executive function deficiencies. The gut microbiome has been linked to cognition. A healthy gut-microbiota-brain axis can be supported by prebiotics to maintain a healthy gut microbiome. Numerous gastrointestinal conditions have been treated by FMT. It has been demonstrated that FMT with *Blastocystis*-positive donors is safe and efficacious in treating recurrent *Clostridium difficile* infection in the *Blastocystis* context, without eliciting unfavorable gastrointestinal symptoms[59,178,179].

According to recent research, changes in the amounts of both good and harmful gut bacteria may be linked to *Blastocystis* infection. The study conducted by Behboud *et al*. revealed that individuals with *Blastocystis* had significantly higher mean relative abundances of harmful bacteria (*Escherichia coli*), beneficial bacteria (*Bifidobacterium, Lactobacillus/Enterococcus*) compared to a control group. Conversely, the relative abundances of *Bacteroides fragilis* and *Enterococcus* were significantly lower[180]. Deng & Tan demonstrated the advantageous effects of *Blastocystis* ST4 on intestinal commensal bacteria using *in vitro* research, encouraging the proliferation of advantageous bacteria like *Bifidobacterium* and *Faecalibacterium prausnitzii*[177]. Increased bacterial diversity and the enrichment of specific taxa, like *Clostridiales* and *Bacillota*, in the gut microbiome have been associated with *Blastocystis* ST4. It has been demonstrated that *Blastocystis* ST4 improves gastrointestinal health by reorganising the gut microbiota and growing the number of helpful bacteria[179]. It has been discovered that *Blastocystis* ST4 stimulates Th2 immune responses and raises the expression of cytokines, including TNFα and IFNγ, which are critical for preserving gut health. *Blastocystis* ST4 has been associated with the stability of the gut microbiota by encouraging the growth of good bacteria like *Shigella* and *Escherichia,* as well as *Akkermansia* and *Lachnospiraceae NK4A136* group. It also prevents the spread of harmful bacteria like *Bacteroides* and *Escherichia*[181].

Using *Blastocystis hominis* as a model, the efficacy of probiotics against parasite infections was verified. Stool samples were taken from children who had frequent diarrhoea as part of an experimental investigation. Isolated *Blastocystis hominis* was utilised to infect BALB/C mice after all stool samples were examined under a microscope for the presence of *Blastocystis hominis* and other parasites. The efficacy of metronidazole combined with probiotics was compared to that of metronidazole and probiotics alone in experimentally infected mice. The mean number of cysts in the stools of infected mice considerably decreased in the metronidazole combined probiotics treatment group as compared to the infected group treated with probiotics and the infected group treated with metronidazole[182]. In another study, Lepczyńska & Dzika assess the role of intestinal microorganisms such as *Escherichia coli, Candida albicans,* and *Candida glabrata* in protozoan proliferation, as well

as the effectiveness of the lactic acid bacteria *Lactobacillus rhamnosus*, *Lactococcus lactis*, and *Enterococcus faecium* in eliminating *Blastocystis* ST3. *In vitro*, the aforementioned bacteria and their cell-free supernatants were co-cultured at varying doses with *Blastocystis* xenic and axenic culture. Both investigations, using xenic and axenic cultures, demonstrated that *Lactobacillus lactis* and *Lactobacillus rhamnosus* inhibited *Blastocystis*. The results of this study suggest that *Lactobacillus rhamnosus*, *Lactobacillus lactis*, and *Enterococcus faecium* may be used as an extra therapeutic regimen in addition to conventional medications or as a preventative measure against *Blastocystis* colonisation[163].

Probiotic bacteria may prevent gut protozoans from multiplying, while some may be advantageous to their growth. Regarding this, *Saccharomyces boulardii* is a notable probiotic that has demonstrated effectiveness against a number of intestinal infections, including *Blastocystis*[183]. In a randomized clinical trial, the treatment rates of *Saccharomyces boulardii* were 94.4% in symptomatic children with *Blastocystis*-positive feces, compared to 73.3% in the metronidazole-treated group[184]. Results from a different study showed that live *Saccharomyces boulardii* considerably enhanced the histological features of intestinal mucosa. In comparison to other therapies, the co-administration of live *Saccharomyces boulardii* and metronidazole produced better efficacy and showed a 100% reduction of the parasite in both the stool and intestinal wash fluid[185]. Additionally, an *in vitro* investigation demonstrated the effectiveness of the lactic acid bacteria, *viz., Lactobacillus rhamnosus*, *Lactococcus lactis*, and *Enterococcus faecium* in the elimination of *Blastocystis*[186].

In a single-blind, randomised trial, the probiotic *Saccharomyces boulardii* and the antibiotic metronidazole were found to be equally effective in treating symptomatic *Blastocystis* infections in children. At 15 days, the probiotic group had a 77.7% clinical cure rate while the metronidazole group had a 66.6% clinical cure rate. Clinical cure rates at 30 days were 94.4% for the probiotic group and 73.3% for the metronidazole group, indicating that probiotics might be a more successful treatment than the typical course of antibiotics[187]. According to *in vitro* research, some probiotic strains, such as *Lactobacillus rhamnosus* and *Lactococcus lactis*, can stop *Blastocystis* from growing by generating lactic acid. Following initial stimulation, other probiotics such as *Enterococcus faecium* and *Escherichia coli* also showed inhibitory effects on *Blastocystis* growth. In the clinical trial, the probiotic *Saccharomyces boulardii* was well tolerated and showed no side effects. On the other hand, metronidazole therapy may not always be the best choice due to the possibility of medication resistance, reinfection, and adverse drug reactions[141]. In order to treat *Blastocystis hominis*, Ghaib et al. assessed the efficacy of *Lactobacillus acidophilus* as a probiotic at different incubation times, bacterial dilutions, and statuses. The results showed that *Lactobacillus*

acidophilus may inhibit *Blastocystis* activity and lower the count with highly significant differences. Axianic cultures also demonstrated *Blastocystis* inhibition.

Non-pathogenic yeast *Saccharomyces boulardii* is sold as a dietary supplement and has shown promise in treating gastrointestinal disorders with a predominantly inflammatory component, suggesting that it may interfere with cellular signalling pathways. It influences local and systemic immune responses, stabilises the functions of the gastrointestinal barrier, induces enzymatic activity that promotes absorption and nutrition, controls intestinal microbial balance, and prevents pathogens from colonising and infecting the mucosa[188]. Dinleyici *et al*. compared the natural evolution of *Blastocystis hominis* infection with the efficacy of *Saccharomyces boulardii* and metronidazole in children with gastrointestinal symptoms. In this randomized single-blinded trial, children with confirmed *Blastocystis hominis* were divided into three groups: *Saccharomyces boulardii*, metronidazole, and no treatment. By day 15, clinical cure rates were 77.7% for *Saccharomyces boulardii*, 66.6% for metronidazole, and 40% for no treatment. Cyst disappearance rates were highest in the metronidazole group (80%), followed by *Saccharomyces boulardii* (72.2%). After one month, clinical and parasitological cure rates were comparable between *Saccharomyces boulardii* and metronidazole groups. The study suggests both treatments are effective[189]. Xia *et al*. (2021) examined the impact of supplementing a corn-based diet with inulin (1, 2, or 4%) or bacitracin (400 ppm) on the caecal microbiota of broiler chickens. Using 16S rRNA sequencing and transcriptomic analysis, they found that *Bacteroides* were the dominant carbohydrate-metabolizing organisms. Inulin and bacitracin did not significantly alter microbiota composition or diversity, but inulin, particularly at 2%, enhanced the activities of *Bacteroides*, *Prevotella*, and *Bifidobacterium*, and 2% level appears to be the most optimal dosage for *Bifidobacteria* activity[190]. Table 5 summarizes the various prebiotics, probiotics, and postbiotics along with their mechanism of action in improving the gut microbiome in *Blastocystis*.

Table 5. Various prebiotics, probiotics, and postbiotics along with their mechanism of action in improving gut microbiome in *Blastocystis*.

Compounds	**Mechanism**	**References**
Prebiotics		
Inulin (FOS)	Inulin promotes a healthy gut microbiome by feeding beneficial bacteria, enhancing their growth, and outcompeting *Blastocystis*, reducing its prevalence.	[191]
Galacto-oligosaccharides (GOS)	Galacto-oligosaccharides boost beneficial gut bacteria, improving microbiome balance, which helps suppress *Blastocystis* growth and mitigate its effects.	[191]

Fructo-oligosaccharides (FOS)	Fructo-oligosaccharides stimulate beneficial bacteria growth in the gut, enhancing microbiome balance and outcompeting *Blastocystis*, reducing its impact	[191]
Fiber-rich foods (oats, wheat, barley, legumes, nuts, seeds)	Fiber-rich foods nourish beneficial gut bacteria, enhancing microbiome diversity and balance, which helps suppress and manage *Blastocystis* overgrowth.	[191,192]
Probiotics		
Lactobacillus rhamnosus	*Lactobacillus rhamnosus* enhances gut microbiome health by increasing beneficial bacteria, inhibiting *Blastocystis* growth, and strengthening gut barrier function.	[141,191]
Lactococcus lactis	*Lactococcus lactis* boosts beneficial gut bacteria, enhances microbiome balance, produces antimicrobial substances, and inhibits *Blastocystis* growth and colonization.	[141,191]
Enterococcus faecium	*Enterococcus faecium* enhances beneficial gut bacteria, produces antimicrobial peptides, and inhibits *Blastocystis* colonization, promoting a balanced microbiome.	[141,191]
Saccharomyces boulardii	*Saccharomyces boulardii* inhibits pro-inflammatory cytokines and inducible nitric oxide synthase genes, promoting a healthy gut microbiome to treat *Blastocystis*.	[141,191]
Postbiotics		
Short-chain fatty acids (SCFAs)	SCFAs promote a healthy gut microbiome by feeding beneficial bacteria, modulating gut microbiota composition, enhancing gut epithelial growth, and reducing inflammation.	[141,192]
Nitric Oxide (NO)	NO inhibits *Blastocystis* growth by downregulating inducible nitric oxide synthase genes and promoting beneficial gut bacteria.	[141,192]
Organic compounds (garlic, ginger, turmeric, etc.)	Organic compounds like garlic, ginger, and turmeric inhibit *Blastocystis* growth by producing postbiotics, modulating gut microbiota, and enhancing gut barrier function.	[141,192]

While the evidence supports the potential of prebiotics and probiotics, particularly *Saccharomyces boulardii*, *Lactobacillus rhamnosus*, and inulin, as adjunct or alternative therapies for *Blastocystis* infection, several important caveats remain. First, most studies are limited by small sample sizes, heterogeneous experimental designs, and limited human clinical data, making it difficult to generalize findings. Additionally, the dualistic nature of *Blastocystis*, acting as both a possible pathogen and a commensal, adds complexity to probiotic treatment strategies, as certain subtypes (e.g., ST4) may actually support gut homeostasis. Strain-specific effects of probiotics and their interactions with individual host microbiota profiles remain underexplored, limiting the ability to tailor interventions

effectively. Furthermore, while co-administration with antibiotics like metronidazole may improve outcomes, the mechanisms behind synergy or resistance are poorly defined. Future research should prioritize large-scale, controlled human trials, focus on the subtype-specific response to probiotics, and elucidate the immunomodulatory and metabolic pathways involved. Understanding these dynamics will be key to developing precision microbiota-targeted therapies for *Blastocystis* and related gut dysbiosis conditions.

7.2. Natural Products with Antiparasitic or Gut-modulating Properties

The protozoan *Blastocystis* causes blastocystosis, an infection that can be treated using natural compounds that have antiparasitic and gut-modulating qualities. Natural therapies are a great substitute for conventional medicines because conventional ones, like antibiotics, frequently have problems like resistance and side effects. In addition to having strong antiparasitic properties, these products—which include herbs like garlic and oregano oil—also promote gut health by regulating microbiota and boosting immune responses[193]. Including these natural products in treatment plans can offer a comprehensive, non-invasive way to manage and possibly even cure blastocystosis.

It has been discovered that the antiparasitic qualities of garlic and ginger prevent *Blastocystis* from growing and becoming viable *in vitro*. It has been demonstrated that the bioactive ingredients in garlic and ginger, such as allicin and gingerols, block the formation of proteins, nucleic acids, and parasite enzymes[192,194]. Strong *in vitro* anti-*Blastocystis* activity has been shown by extracts from plants such as *Artemisia fragrantissima*, *Echinops spinosus*, and *Achillea judaica*, especially against certain subtypes like ST1 and ST3. Some plant extracts exhibit more efficacy against specific subtypes of *Blastocystis*, and they can all reduce the growth and viability of the bacterium. Studies have shown that certain spices, such as cumin, horseradish, and turmeric, have antibacterial properties against *Blastocystis*. These spices' active ingredients, like turmeric's curcumin, can interfere with *Blastocystis* growth and metabolism[192,194]. Plants that contain berberine (such as goldenseal and barberry) and/or oil of oregano, grapefruit seed extract, wormwood, black walnut, and other berberine-containing plants may help suppress intestinal parasite growth, reduce inflammation, support gut health, and improve digestion. A nutrient-rich, anti-inflammatory diet can support parasite management and ease digestive issues, with an emphasis on whole foods such as fruits, vegetables, lean proteins, healthy fats, and high-fiber options. Steer clear of processed and sugary meals because they can exacerbate stomach symptoms, weaken the immune system, and cause inflammation[191].

Experimental investigation of a number of medicinal plant extracts against *Blastocystis* infection has been conducted in comparison with existing therapeutic options[195]. In one study, children with *Blastocystis* infection had a higher cure rate (55.5% and 43%) when treated with nitazoxanide combined with garlic than when treated with nitazoxanide alone (57.7% and 40%)[160]. On the contrary, the investigation of *in vitro* anti-*Blastocystis* efficacy of traditional Chinese medicinal herbs, namely *Brucea javanica* and *Coptis chinensis,* showed less efficacy when compared to metronidazole at comparable concentrations[196]. Additionally, it has been demonstrated that dietary management techniques, including introducing a high-fiber and lactose-free diet, reduce clinical symptoms[197]. In addition, when compared to metronidazole as the standard drug, *Brucea javanica, Nigella sativa, Quercus infectoria, Ferula asafoetida*, *Achillea millefolium, Eurycoma longifolia*, and *Mallotus oppisitifolius*, have also demonstrated anti-*Blastocystis* efficacies[195].

The number of *in vitro* and *in vivo* trials evaluating the anti-*Blastocystis* efficacies of certain native plants has increased recently. The chemical makeup and antibacterial activity of the plants were taken into consideration. The antibacterial effect of garlic (*Allium sativum*) is attributed to a variety of thiosulfinates, such as allicin, which inhibit enzymes in microbes, including thiols. Additionally, garlic's allicin inhibits the parasites' ability to synthesize DNA and proteins and completely prevents them from synthesizing RNA. Furthermore, a component of ginger (*Zingiber officinale*) called hexahydrocurcumin may be useful in eliminating the parasites[198,199]. Yakoob *et al*. determined the growth pattern and *in vitro* susceptibility of *Blastocystis hominis* to various treatments, stool specimens of 16 IBS patients, and 10 controls. Susceptibility assays with metronidazole, garlic, ginger, white cumin, and black pepper were conducted. *Blastocystis hominis* from IBS showed varying responses: metronidazole at 0.01 mg/ml inhibited 38% growth, while garlic at 0.01 mg/ml inhibited 44% growth. Higher concentrations of metronidazole and garlic further reduced growth. *Blastocystis hominis* from controls were more susceptible to treatments. Genotype analysis showed that type 3 and coinfections grew better. Overall, garlic showed notable efficacy, especially against genotype 1 *Blastocystis hominis* from IBS[200].

The pathogenic role of *Blastocystis* in humans and drug resistance issues have spurred interest in natural treatments. Abdel-Hafeez *et al*. evaluated the *in vivo* effects of garlic and ginger on mice infected with *Blastocystis*. Garlic, ginger, and nitazoxanide significantly reduced cyst shedding and improved histopathological outcomes compared to untreated mice. Additionally, infected mice showed elevated nitric oxide and malondialdehyde levels, which were significantly reduced by treatment. Garlic and ginger also demonstrated

antioxidant properties, reducing nitric oxide and malondialdehyde levels effectively. The study concludes that garlic and ginger are beneficial for treating blastocystosis[201]. Another study by Abdel-Hafeez et al. evaluated the *in vitro* susceptibility of *Blastocystis hominis* to nitazoxanide, garlic, ginger, onion, and turmeric. Fecal samples from IBS patients were cultured and treated with 0.1 mg/ml of each extract. After 48 hours, nitazoxanide significantly reduced parasite numbers by 93.33%, followed by ginger (92.98%) and garlic (92.44%). Onion and turmeric had insignificant effects. The study suggests that nitazoxanide, garlic, and ginger effectively reduce *Blastocystis hominis in vitro*[202]. *Blastocystis* is susceptible to nitric oxide, which is important for homeostasis and host defense but can also cause cellular damage, breakdown of the gut barrier, and be involved in the pathogenesis of many inflammatory and autoimmune diseases. Treatments with garlic and ginger significantly reduced the amount of nitric oxide released into the gut, which may have been brought on by a reduction in the amount of *Blastocystis* in the intestine. Downregulation of nitric oxide and malondialdehyde is a key mechanism of garlic- and ginger-induced antiparasitic effects[203,204].

Asafoetida, derived from *Ferula* species, was evaluated for its *in vitro* activity against *Blastocystis* subtype 3. Asafoetida powder (Ap) and oil (Ao) extracts, at varying concentrations, were tested over 24, 72, and 144 h and compared to metronidazole. Both Ap and Ao decreased *Blastocystis* counts and viability, with complete inhibition at 16 mg/ml for Ap and 40 mg/ml for Ao. This inhibitory effect was confirmed by microscopy and was dependent on concentration and incubation time. Asafoetida prevented parasite regrowth in asafoetida-free medium, suggesting its potential as a potent natural alternative for treating *Blastocystis* infection[205]. In Malaysia, herbal plants like *Eurycoma longifolia* (Tongkat Ali) are known for their therapeutic properties. A study screened the anti-protozoal activity of various herbal extracts against *Blastocystis* subtype 3. Tongkat Ali showed the highest efficacy, with its ethyl acetate fraction performing best at 1.0 mg/ml against ST1 (94.9%), ST3 (95.1%), and ST5 (94.3%). Compared to allopathic drugs, metronidazole had the highest activity. This study described Tongkat Ali's anti-protozoal properties against *Blastocystis*, demonstrating significant cell count reduction and uniform sensitivity across subtypes, comparable to metronidazole[206].

In 2009, Vital and Rivera demonstrated that at 0.5 and 1.0% concentrations, ethanol extracts of *Chromolaena odorata* leaves and ethyl acetate extracts of *Uncaria perrottetii* stem bark suppressed *Blastocystis* development and reduced cell counts[207]. The *in vitro* effectiveness of two native plants, *Quercus infectoria* (Fagaceae) and *Achillea millefolium*, which have been used to treat diarrhea in Anatolia, against *Blastocystis*, was investigated

using hexane and methanol extracts. The plant extracts' 50% lethal concentration (LC_{50}) and half-maximal effective concentration (EC_{50}) values were ascertained using the Brine Shrimp and Graphpad Prism 5® techniques, in that order. The methanol extract of *A. millefolium* had the lowest LC_{50} (500 μg/ml) and EC_{50} (198.8 μg/ml) values when compared to other extracts. Its anti-*Blastocystis* activity was found to be similar to metronidazole, and it exhibited no cytotoxic effect[208].

The effect of *Nigella sativa* aqueous extract was evaluated against two isolates of the intestinal protozoan parasite *Blastocystis hominis*. Different concentrations ranging from 10 to 500 μg/ml were tested alongside metronidazole in culture media at 37°C. The extract at 100 and 500 μg/ml showed a potent lethal effect on both isolates. On the 6th day, the inhibitory effect of *Nigella sativa* was comparable to metronidazole. At 500 μg/ml, it significantly reduced the living cell rate. These results suggest that *Nigella sativa* extract could be effective in treating *Blastocystis hominis*[209]. A study evaluated the anti-*Blastocystis* activity of 24 plant parts from 21 medicinal plants from Ghana, traditionally used for stomach disorders. Plants were collected in Greater Accra and tested as ethanolic, warm, and cold water extracts. Six ethanolic extracts showed significant anti-*Blastocystis* activity, with *Mallotus oppositifolius* showing nearly as much efficacy as metronidazole. *Clausena anisata* was the only extract with antimicrobial activity[210]. The activities of n-hexane, dichloromethane, and methanol extracts from five anti-diarrheic Thai medicinal plants were tested against *Blastocystis hominis*. Dichloromethane and methanol extracts from *Brucea javanica* seed and the methanolic extract of *Quercus infectoria* nut gall showed the highest activity. At 2000 μg/mL, these extracts killed 82%, 75%, and 67% of *Blastocystis hominis* and inhibited 94%, 100%, and 76%, respectively. Metronidazole, at 40 μg/mL, killed 97% and inhibited all samples at 1.25 to 20 μg/mL, serving as the reference antiprotozoan drug[211]. Mediterranean oregano oil of *Oreganum vulgare* was administered orally to fourteen adult patients who tested positive for enteric parasites, *Blastocystis hominis*, *Entamoeba hartmanni*, and *Endolimax nana* in their stools. Following a 6-week regimen of 600 mg of emulsified oil of oregano per day, *Blastocystis hominis* in eight instances, *Endolimax nana* in one, and *Entamoeba hartmanni* in four cases completely vanished. Of the 11 patients who tested positive for *Blastocystis hominis*, seven saw an improvement in their gastrointestinal problems[212]. A recent study investigated the *in vitro* activity of natural substances against *Blastocystis* subtypes ST3 and ST7. Garlic and turmeric were most effective against ST3, while horseradish and turmeric were most effective against ST7. The extracts showed potent antimicrobial activity, with 50% inhibitory concentration (IC_{50}) values ranging from 3.3 to 72.0 μg/ml for ST7 and 3.8 to 4.8 μg/ml for ST3. The study confirmed that ST7 is more resistant to plant extracts than ST3, complicating its eradication[194]. The antiprotozoal and

antibacterial effectiveness of carvacrol, Cassia oil, a combination of thyme and rosemary essential oil, and *Quillaja saponaria* saponin against *Histomonas meleagridis*, *Tetratrichomonas gallinarum*, and *Blastocystis* were assessed in a recent investigation. For five *Histomonas* isolates, the minimal lethal concentrations (MLCs) of carvacrol, the essential oil combination, and the saponin were comparable. The MLCs of the cassia oil ranged from 0.25 to 0.50 µl/ml. The MLCs of the *Blastocystis* isolates varied for every drug, whereas *Tetratrichomonas gallinarum* exhibited the same susceptibilities[213]. Table 6 summarizes the anti-*Blastocystis* activity of various natural compounds, including their effects on different strains and subtypes, and the outcomes observed in both *in vitro* and *in vivo* studies.

Natural products present a promising and increasingly studied alternative or adjunct to conventional drug therapies against *Blastocystis* infection, particularly in light of rising antimicrobial resistance and adverse drug effects. Multiple herbs and plant-derived compounds, including garlic, ginger, turmeric, *Nigella sativa*, and *Tongkat Ali*, have demonstrated notable *in vitro* and *in vivo* efficacy, with some producing comparable effects to metronidazole. These compounds frequently exhibit additional benefits, such as antioxidant, anti-inflammatory, and gut-modulating properties, making them appealing for managing blastocystosis holistically. However, significant challenges persist. Most studies remain preliminary, with a heavy reliance on *in vitro* models and limited standardization of extract preparation, concentration, and delivery. Furthermore, efficacy often varies by *Blastocystis* subtype, highlighting the need for subtype-specific testing and personalized approaches. The absence of large-scale clinical trials, inconsistent methodologies, and poorly understood pharmacodynamics limit the translational potential of these findings. Future research should focus on elucidating mechanisms of action, conducting well-controlled human studies, and identifying synergistic effects between natural compounds and existing antiparasitic drugs. Additionally, better characterization of host-parasite-microbiota interactions is crucial to leveraging these natural therapies in a precision medicine framework.

Table 6. Anti-Blastocystis activity of various natural compounds against different strains and observed outcomes.

Compound		**Strain**	**Outcome**	**References**
Natural/ plant	**Standard**			
Garlic	Metronidazole	*Blastocystis hominis*	Metronidazole at 0.01 mg/ml inhibited 38% growth, while garlic at 0.01 mg/ml inhibited 44% growth. Higher concentrations of metronidazole and garlic further reduced growth.	[200]

Garlic and ginger	Nitazoxanide	*Blastocystis*	Garlic, ginger, and nitazoxanide significantly reduced cyst shedding ($P \leq 0.001$, 0.0001, 0.0003, respectively) and improved histopathological outcomes compared to untreated mice.	[201]
Garlic, ginger, onion, and turmeric	Nitazoxanide	*Blastocystis hominis*	After 48 hours, nitazoxanide significantly reduced parasite numbers by 93.33% ($P < 0.001$), followed by ginger (92.98%, $P < 0.002$) and garlic (92.44%, $P < 0.002$). Onion and turmeric had insignificant effects ($P < 0.15$ and $P < 0.22$).	[202]
Asafoetida powder (Ap) and oil (Ao)	Metronidazole	*Blastocystis* subtype 3	Both Ap and Ao decreased *Blastocystis* counts and viability, with complete inhibition at 16 mg/ml for Ap and 40 mg/ml for Ao.	[205]
Eurycoma longifolia (Tongkat Ali)	Metronidazole	*Blastocystis* subtype 3	Tongkat Ali showed the highest efficacy, with its ethyl acetate fraction performing best at 1.0 mg/ml against ST1 (94.9%), ST3 (95.1%), and ST5 (94.3%)	[206]
Chromolaena odorata leaves, *Uncaria perrottetii* stem	-	*Blastocystis*	0.5 and 1.0% concentrations, ethanol extracts of *Chromolaena odorata* leaves and ethyl acetate extracts of *Uncaria perrottetii* stem bark suppressed *Blastocystis* development and reduced cell counts.	[207]
Quercus infectoria (Fagaceae) and *Achillea millefolium*	Metronidazole	*Blastocystis*	The methanol extract of *Achillea millefolium* had the lowest LC_{50} (500 μg/ml) and EC_{50} (198.8 μg/ml) values when compared to other extracts and have similar efficacy as metronidazole.	[208]
Nigella sativa	Metronidazole	*Blastocystis hominis*	Aq. extract at 100 and 500 μg/ml showed a potent lethal effect on both isolates. On the 6th day, the inhibitory effect of *Nigella sativa* was comparable to metronidazole	[209]
Mallotus oppositifolius	Metronidazole	*Blastocystis*	Same efficacy as metronidazole	[210]
Brucea javanica seed	Metronidazole	*Blastocystis hominis*	At 2000 μg/mL, dichloromethane and methanol extracts killed 82%, 75% and inhibited 94%, 100% respectively.	[211]
Quercus infectoria nut gull	Metronidazole	*Blastocystis hominis*	At 2000 μg/mL, methanolic extract killed 67% of *Blastocystis hominis* and inhibited 76%.	[211]
Mediterranean oregano oil of	Metronidazole	*Blastocystis hominis*	Following a 6-week regimen of 600 mg of emulsified oil of oregano per day, *Blastocystis hominis* in eight instances vanished completely.	[212]

Oreganum vulgare				
Garlic and turmeric	Metronidazole	*Blastocystis* subtype ST3	IC_{50} ranging from 3.8 to 4.8 μg/ml.	[194]
Horseradish and turmeric	Metronidazole	*Blastocystis* subtype ST7	IC_{50} ranging from 3.3 to 72.0 μg/ml.	[194]

7.3. Fecal Microbiota Transplantation (FMT) as a Potential Future Option

FMT involves transferring fecal material from a healthy donor into a patient's gastrointestinal tract. Initially developed for recurrent *Clostridioides difficile* infections, FMT restores healthy gut microbiota balance in various gastrointestinal disorders[214]. FMT can be administered through nasogastric/nasojejunal tube, upper endoscopy, retention enema, or colonoscopy, achieving success rates over 90% for recurrent *Clostridioides difficile* infections[215]. FMT has expanded to conditions such as IBD and IBS[216]. Beyond *Clostridioides difficile* infections, FMT shows potential in managing gastrointestinal disorders by addressing dysbiosis[217,218]. FMT is also explored for metabolic diseases, autoimmune diseases, tumors, and nervous system diseases[219].

FMT reintroduces a diverse microbial community from healthy donor fecal into the patient's gut, restoring normal gut microbiota composition disrupted by chronic infections or inflammatory diseases[220]. It works by outcompeting pathogenic microorganisms, enhancing immune function, improving mucosal barrier integrity, and reducing inflammation[221,222]. FMT enhances beneficial microbes, increases microbiome diversity, and restores normal flora, though exact mechanisms are not fully understood[221,223]. Research shows sustained improvements in microbial diversity and function post-FMT, contributing to long-term health benefits[224]. FMT also modulates immune responses, impacting gut microbiota-host interactions, and immune responses[225].

FMT is explored as a potential treatment for *Blastocystis* infections, reducing symptoms and eliminating *Blastocystis* in patients unresponsive to conventional therapies[226]. Experimental colonization with *Blastocystis* ST4 in colitis mouse models promotes protective immune responses and modulates the gut microbiome, suggesting potential benefits[227]. Similarly, one study found that human transmission of *Blastocystis* through FMT did not cause gastrointestinal symptoms, achieving an 84% success rate in treating 31 recurrent *Clostridioides difficile* infection patients[226]. *Blastocystis*-infected animals exhibit non-inflammatory colonic hypersensitivity and increased serine protease activity, linking *Blastocystis* infections, dysbiosis, and behavioral alterations[228]. This

finding is supported by her who linked fecal dysbiosis in chronically *Blastocystis*-infected rats to colonic hypersensitivity and behavioral alterations.

Despite its success, gaps remain in understanding gut microbiota behavior, necessitating further research[229]. Mechanistic investigations into FMT, especially for *Clostridioides difficile* infections, highlight its importance as a complementary or alternative treatment. Future FMT research aims to optimize donor selection, fecal processing, and delivery methods to enhance efficacy and safety[230,231]. Personalized FMT approaches based on individual microbiome profiles may improve outcomes[232]. Ongoing research evaluates these innovations to establish best practices for FMT in diverse clinical contexts[233]. FMT is effective for recurrent *Clostridioides difficile* infections, with a success rate of 84% for *Blastocystis*-containing fecal suspensions[230]. FMT restores disrupted microbiota, addressing imbalances and highlighting its growing therapeutic interest.

7.4. Nanotechnology-assisted Drug Delivery

Nanotechnology has significantly advanced drug delivery systems by enabling the manipulation of materials at the nanoscale, leading to the development of advanced nanocarriers such as liposomes, nanoparticles, and dendrimers. These nanocarriers encapsulate therapeutic agents and transport them precisely to specific sites of infection, enhancing drug delivery in terms of targeting, bioavailability, and reducing side effects[234]. Nanocarriers come in various forms, including polymer-based, lipid-based, viral, and inorganic nanoparticles, each offering unique advantages in drug delivery[235]. They have demonstrated successes in targeted drug delivery by improving bioavailability, increasing drug loading capacity, enhancing intracellular delivery, and ultimately improving therapeutic effects[236]. Nanocarriers have shown significant promise in the treatment of various diseases, including cancer, by offering targeted drug delivery options that can improve treatment outcomes[237]. The integration of natural products with nanodrug delivery systems has further expanded the therapeutic potential of these approaches in combating complex diseases such as cancer and parasitic infections[238]. Nanocarriers in drug delivery systems have been particularly beneficial in overcoming barriers such as the blood-brain barrier, enabling the delivery of therapeutic agents to the central nervous system and solid tumors[239]. The development of smart nanocarriers, such as thermosensitive nanocarriers and magnetite nanoparticles, has enabled controlled drug delivery through mechanisms such as magnetic control and pH-responsive release, offering precise and efficient drug delivery options[240,241]. These advancements in nanocarrier technology have paved the way for multifunctional and stimuli-responsive nanocarriers that can further enhance targeted therapeutic delivery[236].

Nanotechnology offers a promising approach for targeting *Blastocystis* infections with enhanced precision through the development of specialized nanocarriers. These nanocarriers can be tailored to improve the solubility and stability of antiparasitic drugs, enhance their absorption in the gastrointestinal tract, and deliver them directly to infected cells, thereby maximizing drug efficacy while minimizing off-target effects and potential side effects[242]. The utilization of nanotechnology in drug delivery systems provides a targeted approach to effectively combat *Blastocystis* infections. By utilizing nanocarriers, drug delivery can be optimized to enhance the efficacy of antiparasitic drugs while minimizing adverse effects[234,236]. Nanocarriers, such as liposomes, nanoparticles, and dendrimers, serve as a platform for encapsulating therapeutic agents and delivering them precisely to the site of infection, thereby improving drug bioavailability and reducing side effects[234,235]. Moreover, the development of smart nanocarriers, such as thermosensitive nanocarriers and magnetite nanoparticles, enables controlled drug delivery mechanisms that respond to specific stimuli, offering precise and efficient drug delivery options[240,241]. Research into specific nanocarrier designs and their interactions with *Blastocystis* is essential for the development of effective treatments customized to combat this infection[243]. Metronidazole remains the most commonly used drug for treating *Blastocystis* infections; however, concerns have arisen regarding treatment failure, adverse side effects, and the emergence of metronidazole-resistant *Blastocystis* strains[242]. Studies have demonstrated that metronidazole resistance can lead to treatment failures, underscoring the necessity for alternative treatment strategies to address drug-resistant isolates of *Blastocystis*[244]. One promising approach involves the use of nanoparticle-based drug delivery systems, which can enhance the efficacy and bioavailability of antiparasitic compounds while potentially reducing toxicity and resistance development.

Nanotechnology has shown promise in treating *Blastocystis* infections through the utilization of nanocarriers to enhance the efficacy of antiparasitic drugs. Gold nanoparticles conjugated with metronidazole have demonstrated improved efficacy in targeting and killing *Blastocystis* in both *in vitro* and animal models. Additionally, silver nanoparticles have proven effective in treating various parasitic infections, including *Blastocystis*[245]. The synthesis of nanoparticles, such as gold and silver nanoparticles, offers a novel approach to developing effective drug candidates for combating parasitic diseases[246]. The use of nanoparticles in drug delivery systems provides a targeted and efficient method of delivering antiparasitic agents, thereby enhancing their effectiveness while minimizing off-target effects[247]. Nanoparticles have been investigated for their potential to deliver antimicrobial agents, including antiparasitic drugs, to combat a range of infectious diseases caused by parasites[248]. These nanocarriers serve as a platform for improving the solubility, stability,

and targeted delivery of drugs, presenting a promising alternative to conventional treatment methods for *Blastocystis* infections.

Future developments in nanotechnology-assisted drug delivery hold the potential to revolutionize the treatment of infections such as *Blastocystis* by introducing innovative strategies. One emerging trend involves the development of stimuli-responsive nanoparticles that can release drugs in response to specific environmental triggers. By utilizing stimuli-responsive nanoparticles, drug release can be precisely controlled, preventing premature release and enhancing therapeutic efficacy[249]. These smart drug delivery systems, incorporating stimuli responsiveness triggered by internal or external stimuli, are considered the future of drug delivery[250]. Another promising trend is the creation of multifunctional carriers capable of delivering multiple therapeutic agents simultaneously. The development of drug delivery systems with multiple functions, such as simultaneous stimuli-responsive drug release and real-time imaging, represents a significant advancement in the field[251]. Moreover, the integration of stimuli-responsive drug delivery systems can prevent premature drug release, a common issue with traditional delivery systems, and enhance therapeutic efficacy while minimizing adverse effects[252]. These systems can be designed to respond to various external stimuli, such as light, temperature, magnetic fields, and ultrasound, providing precise control over drug release[250]. Furthermore, the use of multifunctional and stimuli-responsive magnetic nanoparticle-based delivery systems has garnered interest in the diagnosis and therapy of cancer, showcasing the potential of such systems in targeted drug delivery[253]. These systems offer a multifaceted approach to drug delivery, combining targeting capabilities with stimuli responsiveness to improve treatment outcomes.

The integration of nanotechnology into drug delivery systems represents a transformative advancement in the treatment of parasitic infections such as *Blastocystis*. By enhancing drug solubility, stability, and site-specific targeting, nanocarriers offer a promising strategy to overcome current therapeutic limitations, including drug resistance and systemic side effects. Continued research into the design and optimization of stimuli-responsive and multifunctional nanocarriers could pave the way for more precise, efficient, and patient-friendly treatment regimens. As the field progresses, nanotechnology-based therapies have the potential to significantly improve clinical outcomes for *Blastocystis* infections and set a precedent for addressing other parasitic diseases.

8. Conclusion

In summary, various treatment strategies are available for treating *Blastocystis* infections. Various drugs such as metronidazole, TMP-SMX, and nitazoxanide have been

widely investigated as treatment options for *Blastocystis* infections. However, future studies should further evaluate the relationship between the various *Blastocystis* subtypes and their variations in drug susceptibility. Additionally, it is also imperative to determine their molecular mechanisms of action and potential resistance to each existing drug. Although various alternative treatment strategies such as the use of natural compounds with antiparasitic or gut-modulating qualities, probiotics and prebiotics to support a healthy gut microbiome, and FMT have been extensively studied, it is important to fully assess their safety and effectiveness before incorporating them as a treatment option for *Blastocystis* infections. Furthermore, the potential use of nanotechnology as a delivery method for these medications to improve their safety and effectiveness also requires further validation.

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