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A LATEST DEVELOPMENT IN DIAGNOSIS AND TREATMENT OF BLASTOCYSTIS HOMINIS

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ABSTRACT
Blastocystis is a common intestinal parasite, previously known as Blastocystis hominis, that is found worldwide in both humans and animals. Its role as a pathogen is debated because most infected individuals are asymptomatic, though it is linked to gastrointestinal issues like diarrhea, abdominal pain, and vomiting in some cases. Diagnosis requires finding the organism in a stool sample and correlating it with symptoms. While some infections may resolve on their own, treatment with antibiotics like metronidazole or other antiprotozoal medications may be considered if symptoms are severe. Blastocystis hominis infection, also called blastocystosis, is an infection with a single-celled parasite that is often asymptomatic, but can cause gastrointestinal symptoms like diarrhea, abdominal pain, and gas. Infection is spread through the fecal-oral route, which includes ingesting contaminated food or water and poor hygiene. A microscopic parasite that can be found in the intestines and is being studied for its link to Irritable Bowel Syndrome (IBS), a functional gastrointestinal disorder. Research is ongoing to understand Blastocystis' role, with some studies finding it in individuals with IBS symptoms like diarrhea, bloating, and abdominal pain, while others find it in asymptomatic individuals, indicating the pathogenic potential is still controversial. Treatment for symptomatic cases may involve antibiotics, but diagnosis and treatment can be challenging as many infections are mild and resolve on their own. The prevalence of Blastocystis hominis varies globally, being significantly higher in developing countries (often exceeding 50%) compared to developed countries (typically around 10% or less). Transmission can occur through contaminated food and water, and via animal-to-human and human-to-human contact. Factors like sanitation, socioeconomic conditions, and personal hygiene play a major role in the parasite's prevalence. Blastocystis hominis infection in females is similar to in males, though some studies show slight prevalence differences. Many individuals are asymptomatic, but when symptoms occur, they can include diarrhea, abdominal pain, bloating, gas, and anal itching. Females, particularly pregnant women, can also be at risk for iron deficiency anemia from this infection
Keywords: ***Diagnosis; Transmission; contamination; treatment; Blastocystis hominis***

1. INTRODUCTION

The microscopic parasite *Blastocystis* is able to live in human's digestive canal. The role of this parasaite in causing diseases is not entirely understood by the researchers. Some patients who have this parasaite in their feces suffer from diarrhoea, abdominal pains or other gastrointestinal disorders. Nevertheless, most frequently, blastocystis parasite is basically harmless when it lives in patients' digestive canal. The transmission of Blastocystis may occur via water or food or through contacts with human's or animal's stool. Infection with Blastocystis is usually more commonly observed among individuals living or traveling to developing countries and among those who are in contact with animals. In humans, Blastocystis was once observed as a single species, Blastocystis hominis. Many variations were found by researchers, either different strains or different species within a single species. Nowadays, *Blastocystis spp* is the scientific name which is used as an abbreviation which means "multiple species." and the term blastocytosis is the term used to indicate blastocystis infection.

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The latest data from the World Health Organization (WHO) stated that 12 million cancer global cases and 4 million deaths due to cancer were reported. Of these cancers, colorectal cancer (CRC) ranked the 3rd in terms of morbidities about (2) millions and 2nd in deaths about (1) million [1]. The risk factors for cancer development include chronic inflammations and infections as well as unhealthy diets, stressful lifestyles, cell damage, continuous exposures to radiations in addition to harmful chemicals [2, 3]. About (16%) of cancers are caused by Infectious agents such as parasites [4]. *Blastocystis hominis* and *Cryptosporidium* spp. are the ubiquitous opportunistic protozoa that are isolated from the human's gastrointestinal tracts and considered as infectious factors [5, 6]. Because these enteric prevalent parasites are located in the gastrointestinal canal, they can cause severe challenges to immunocompromised patients who receive colorectal cancer chemotherapy ([4, 7]. *Cryptosporidium hominis* and *Cryptosporidium parvum* are among *Cryptosporidium* species that can cause more than (90%) of the human infections [8]. Until now, from the 22 recognized *B. hominis* subtypes (ST1-ST22), (10) subtypes were isolated from humans (ST1-9 and ST12), and ST3 was the most prevalent subtype [9]. Both parasites are zoonotic and transmitted through oral-fecal route and contaminated foods and water in addition to close contacts with animals [10, 11]. *B. hominis* and *Cryptosporidium spp* infections are usually reported in people with gastrointestinal complications such as abdominal cramp and diarrhea, although their pathogenesis was not obviously recognized [12, 13]. *B. hominis* and *Cryptosporidium* spp. are often controversially identified in healthy individuals in addition to persons with gastrointestinal disorders, without ignoring their risk as opportunistic parasites in patients who receive chemotherapeutic drugs [14, 15]. Until the present time, different studies were done on the pathogenic ability and potential relationship of these two parasites with non-communicable diseases like irritable bowel syndrome, Crohn disease as well as gastrointestinal carcinomas [16,17,18,19]. In the latter, various studies concentrated on the possible virulence roles and prevalence of these parasites in causing colorectal cancer. This meta-analysis and systematic review is planned and accomplished aiming to aggregate the available data and provide a comprehensive and statistically-documented representation of pooled prevalence and odd ratios (OR) of both *B. hominis* and *Cryptosporidium* spp. Among patients with CRC and their potential relationship with this fatal cancer [20,21].

The microorganism, Blastocysts, is single-celled protozoan. Several protozoans usually live in human's digestive system and cause no harm or even are helpful parasites, while other parasites can cause diseases [22]. It was mentioned that 32 subtypes of *Blastocystis hominis* are present. The clinical features in infected people rely upon the subtype which causes the infection. *Blastocystosis* was reported in immunosuppressed individuals e.g. those who underwent kidney transplantations [23,24]. In addition, It was stated that the infection was more frequently observed in healthy persons compared to those with inflammatory bowel diseases [24]. Moreover, according to the literature, it was reported that individuals who have *Blastocystis* in their bowels have an increased intestinal bacterial diversities, indicating that the presence of *these* parasites will cause beneficial impact on the intestinal microbiota [25]. Nonetheless, the impact of *Blastocystis* will also vary according to the infecting subtype, hence *Blastocystis hominis* is still debatable whether it is a commensal or a pathogenic organism due to the very wide genetic variations [26].

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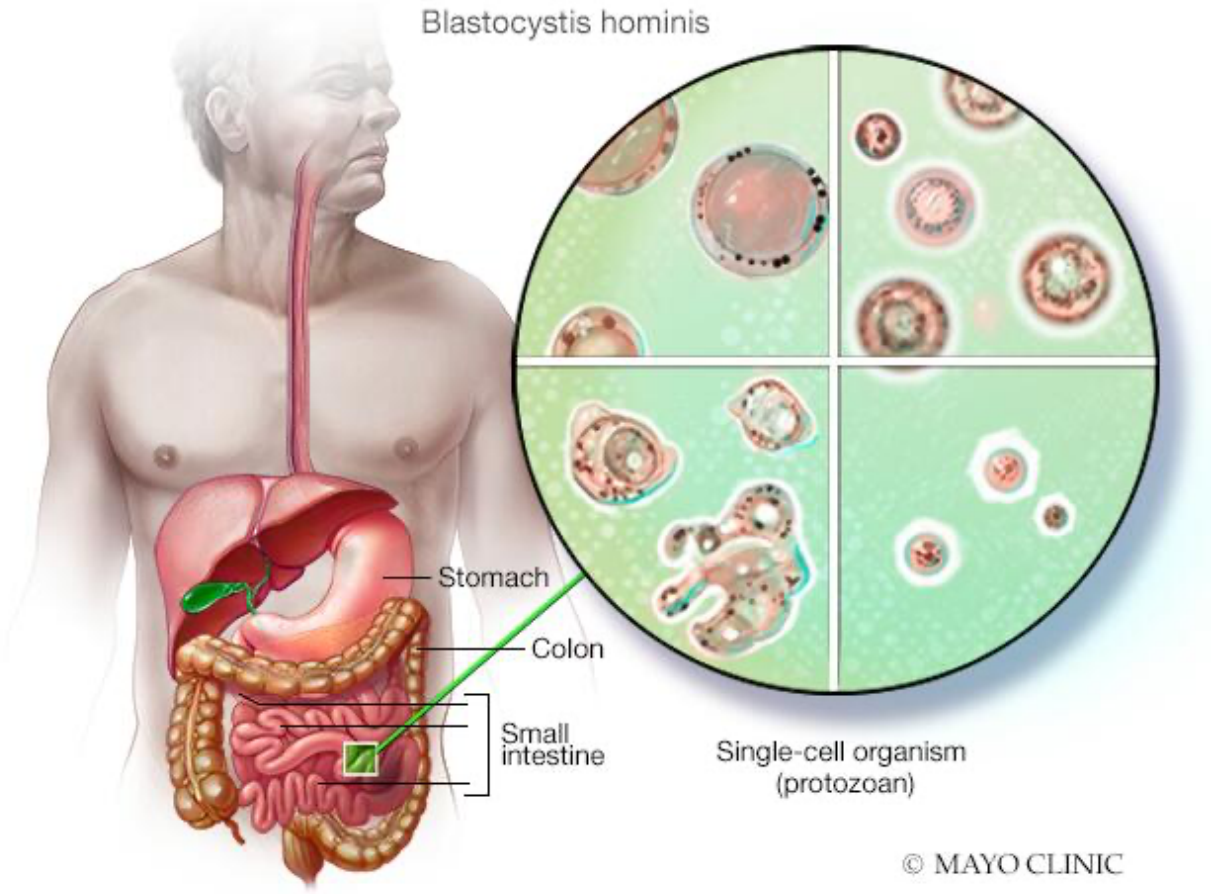

The microorganism, Blastocysts, is single-celled protozoan. Several protozoans usually live in human's digestive system and cause no harm or even are helpful parasites, while other parasites can cause diseases [27,28]. Whether blastocystis causes a disease is not obvious yet. The majority of infected individuals with the parasite are asymptomatic, however, it is also seen in patients suffering diarrhea and other digestive disorders [28].

Complications

If a person suffers from diarrhea due to blastocystosis, it is probably a self-limiting. Nevertheless, when the patient suffers from diarrhea, he loses necessary fluids, minerals and salt, that may result in dehydration. Children are particularly susceptible to dehydrations [29].

2. MATERIALS AND METHODS

The etiology of *Blastocystis hominis* is primarily through the fecal-oral route, with transmission occurring from contaminated food or water or by direct contact with infected feces [30]. Risk factors include poor sanitation, close contact with animals, travel to countries with lower sanitation standards, and poor hygiene practices [31]. Zoonotic transmission from animals is also considered a likelyroute. *Blastocystis* parasites which are isolated from human-beings are usually called *B. hominis*. However, the term *Blastocystis spp.* is regarded more suitable due to wide genetic variety (even among microorganisms isolated from human) and low host specificity [32]. When performing the genetic typing, the subtype (ST) must also be observed according to consensus terminology. The 4 most prevalent subtypes include ST1, ST2, ST3 and ST4 among the 9 human subtypes, while other subtypes occur sporadically and may be associated with zoonotic transmissions [33].

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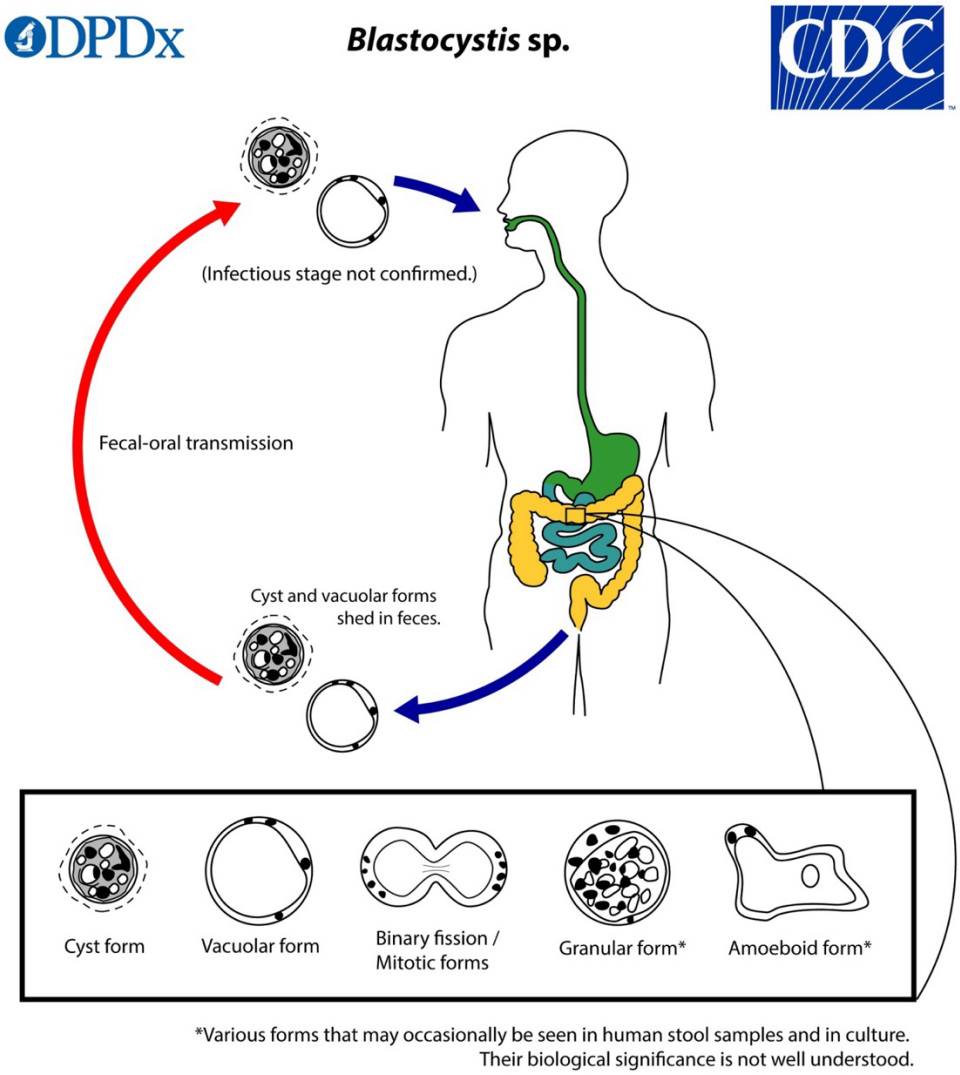

The life cycle of *Blastocystis* spp. has not been yet understood, particularly the parasite's infectious stages and which of the different morphologic form of this parasite which is diagnosed in feces or cultures form the parasite's distinct biologic stage in the host's intestines [34]. The infectious stage of the parasite is supposed to be the (3–5 µm) diameter cyst, although it is not confirmed yet. The major forms found in human's fecal samples is called the vacular or central body with different size from (5–40) µm or much larger [35]. It appears that the parasite's replication occurs through binary fissions. In fecal samples and/or cultures, other morphologic forms such as (ameboid or granular) were also seen.; additional researches are needed to investigate the biological roles and final developmental fates of the parasite [36].

3. RESULTS AND DISCUSSION

Diagnosis of *Blastocystis hominis*

Polymerase chain reaction for *Blactocystis hominis* detection

The PCR thermocycler and GoTaq®Green Master Mix kits (Promega, U.S.A) were used to detect the parasite in frozen fecal specimens in accordance with the guidelines of the manufacturer in a final volume of 25 µl reaction [37]. Two different pairs of primers were utilized to detect the 1770 bp and the 1100bp16S-like ribosomal RNA of *B. hominis* genes,

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with the first pair including the forward BH1 (5TATCTGGTTGA TCCTGC CAGT) & the reverse BH2 (3 TGATCCTTCCGCAGGTTCACCTAC) [38]. has established this pair of primers and also Init *et al*.(13) used it. The PCR condition composed of on cycle that denatured at 94 °C for 5 minutes, 35 cycles that included annealing at 54 °C for 1 second, and extended at 72 °C for 1 second, denatured at 94 °C for 30 seconds, with a further cycle with 5 minute chain elongations at 72 °C. The forward BH3 (5GGAGGTAGTGACAATAAATC 3) and the reverse BH4 (3` CGTTCATGAACAATTAC 5) were included in the second pair and applied in Nested PCR. The PCR condition composed of a single cycle that was denatured at 94 °C for 4 minutes, 35 cycles included annealing at 54 °C for 30 seconds, extended at 72 °C for 30 seconds, denatured at 94 °C for 30 seconds, and a further cycle with 5 minute chain elongations at 72 °C. 1% agarose gel with Tris-boric EDTA buffer (Bio Basic, Canada) were used for the CR product electrophoresis. The UV light was used to visualize the fragment, and the fragment size was confirmed the DNA length standard bands (Promega, U.S.A) [39].

New Approaches to Diagnosis

New approaches for diagnosing *Blastocystis hominis* focus on molecular methods like Polymerase Chain Reaction (PCR), which offers high specificity & sensitivity, and advanced techniques such as Direct Fluorescent Antibody (DFA), providing faster results than traditional culture. These methods are replacing or supplementing traditional microscopy and culture, which can have lower sensitivity due to variable organism shedding [40].

Molecular beacons

Molecular beacons reference points are oligonucleotide which transmit light when a substance response happens. As of late, a novel test that utilizes sub-atomic guides for the fast identification of changes related with drug opposition have been created [41]. The test utilizes fluorescent-marked, clip formed DNA tests where fluorophore is adjoining particle which extinguishes the lights. In a continuous PCR examine, when the intensified PCR items have the ordinary, wild types quality succession, the DNA test unfurls, bringing about distinguishable fluorescence. Safe strains with changes in the objective grouping don't hybridize with the test [42].

The test remains collapsed, with no fluorescence is distinguished. Albeit barely any examinations have assessed atomic reference points, accessible information propose that these tests have high affectability (89–98%) what's more, explicitness (99–100%) for the rifampicin obstruction recognition [43]. Affectability for the isoniazid opposition recognition is lower on account of the variety of changes which may result in protection from isoniazid Sub-atomic reference points are most certainly not accessible as business packs which are not FDA-endorsed. They need sophisticated technologies which limit their widespread uses. Their uses are commonly limited to reference and research laboratories [44].

In humans and different animals, 22 Blastocystis subtypes were identified according to small subunit (SSU) rRNA gene investigations, and these subtypes may represent various species [45]. In former studies, *Blastocystis hominis* infection prevalence appeared to be about 50% in the developing countries [46,47], and reached to about 20% in the developed countries.

Line probe assays.

The ubiquitous parasite *Blastocystis sp.* lives in the intestinal canal of humans and several animals. The major clinical method for its diagnosis is the microscopic investigation which is subject to false negative results. For prevention and controlling blactocystosis, it is recommended and important to perform a quick and simple diagnosis of this parasite. The recombinase polymerase amplification-lateral flow dipstick (RPA-LFD) technique was established to rapidly and visually detect *Blastocystis sp.* The amplification of DNA can be done at 37 °C within 18 minutes [49]. Minimum DNA detection's limit was 1 pg/μL, with no cross-reaction with other 12 non-target pathogenic microorganisms, which consisted with the conventional PCR sensitivity. In addition, 56 stool specimens from the 3rd Affiliated Hospital

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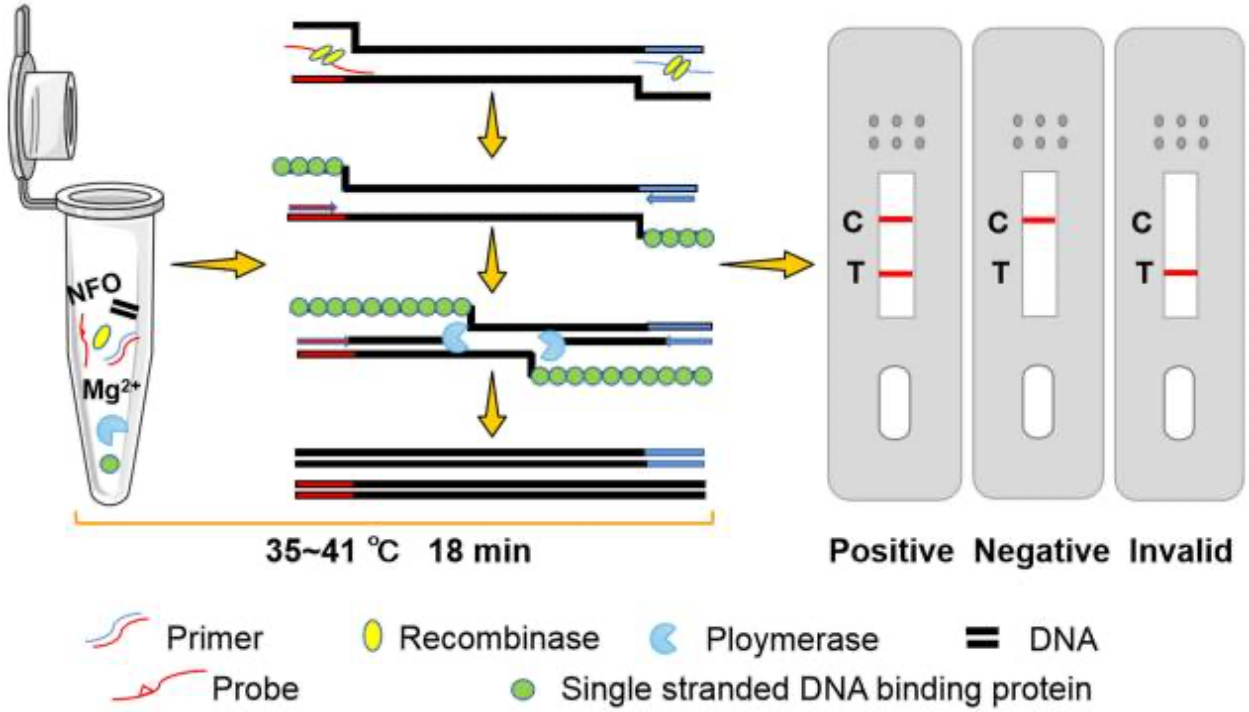
at Xinxiang Medical University have been examined by the use of RPA & cPCR techniques respectively, showing entirely consistent results. It was found that the RPA-LFD assay was highly accurate and the visual findings provided a new choice for rapid field detections and differential diagnosis of *Blastocystis sp.*[50].



Treatment of *Blastocystis hominis*

Treatment for ***Blastocystis hominis*** infection is generally recommended only for individuals with persistent symptoms after other causes have been ruled out. Asymptomatic carriers typically do not require treatment, as the infection may resolve on its own or the organism may be a harmless part of the gut microbiome [51].

If a patient is infected with asymptomatic blastocystis, then he needs no treatment. In few days, mild symptoms and signs will be improved on their own [52].

Possible treatments to eliminate blastocystis and improve its symptoms involve:

- Antibiotics including tinidazoles (Tindamax) metronidazole (Flagyl)
- Combination medications, such as sulfamethoxazole and trimethoprim (Bactrim, Septra, others)
- Anti protozoa treatments e.g. paromomycin or nitazoxanides (Alinia)

There is a great variation between the responses to these treatments. In addition, since the symptoms are not due to the parasite, improvement can be resulted from the treatment's effect on another microorganism [53].

Clinical care of Blastocystoses

Key point

- Clinical importance of *Blastocystis* sp. is controversial.
- Many different treatments were used for *Blastocystis* treatment with different success degrees.
- Safety precautions of treatments in various populations must be considered.

Treatment options

M**etronidazole*** drug treatment at different dosages was reported, e.g. (adult):

Drugs

Example adult's dose

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Durations
Metronidazoles
250 mg – 750 mg oral administration
3x/day for ten day
Metronidazoles
1500 mg oral administration
Once daily for ten day
Note*:* In some areas, lack of response to metronidazoles was reported (Yakoob *et al.,* Br. J. Biomed. Sci. 2004, 61:75).
trimethoprim (TMP)*/sulfamethoxazole (SMX)* drug treatment at different dosages was reported, e.g. in adult:

Drugs
e.g. adult's dosage: Durations
Trimethoprims (TMP) */ sulfamethoxazoles (SMX)*
6 mg/kgs TMP*, 30 mgs/kgs SMX*Once per day for seven day
Trimethoprims (TMP) */ sulfamethoxazoles (SMX)*
320mgs TMP*, 1600 mgs SMX* Once per day for seven day
Trimethoprims (TMP) */ sulfamethoxazoles (SMX)*
160 mgs TMP*, 800 mgs SMX* Two times per day for seven day

Nitazoxanide* treatment appeared to be effective in organism clearing and symptom improvement at the following dosages [54.55]:
Drugs
e.g. dosages: Durations
Nitazoxanides* Adult: 500 mgs, orally two times per day for three day
Nitazoxanides*
Children (4–11) yrs.: 200 mgs, orally two times per day for three day
Nitazoxanides* Children (1–3) yrs.: 100 mgs, orally two times per day for three day
Tinidazoles*, paromomycins*, iodoquinols* and ketoconazoles* was also used to clear *Blastocystis* as shown in the case report or small series [56].
*Note FDA approved for these indications
New Approaches to Treatment
New approaches to ***Blastocystis*** **research focus on advanced molecular techniques**, a **"One Health" integrated view** of human and animal infections, and a reevaluation of its role in the gut as a potential **symbiont rather than strictly a pathogen [57]**.
Diagnostic Advancements

- **Molecular Techniques:** Polymerase Chain Reactions (PCR), especially real time PCR (qPCR) and Next-Generation Sequencing (NGS), have become the gold standard for detection because of their high specificity & sensitivity, surpassing traditional microscopy and culture methods [58].
- **Subtype Identification:** Molecular methods allow for the differentiation of over 40 distinct genetic subtypes (STs), with 16 found in humans (ST1-ST10, ST12, ST14, ST16, ST23, ST35, ST41). This is crucial as different STs may have varying pathogenicity and drug responses [59].
- **"Omics" Technologies:** Metagenomics, metatranscriptomics, proteomics, and metabolomics are used to understand the parasite's metabolic activity and complex interactions within the gut microbiome [60].

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- **Advanced Microscopy & AI:** The application of live-cell imaging and AI-based automated digital imaging is improving the visualization and quantification of different morphological forms (vacuolar, granular, amoeboid, and cystic) [61].

New Understanding of Pathogenesis and Ecology

- **Microbiome Interactions:** Research increasingly suggests that *Blastocystis* presence is related to higher bacterial diversities as well as a healthy intestinal microbiome in many individuals, challenging its classification as an outright pathogen. Some subtypes (ST1, ST3, ST4) may even have beneficial, anti-inflammatory effects [62].
- **Subtype-Specific Effects:** The clinical outcome is highly dependent on the specific subtype and the host's immune status. For instance, ST7 is often linked to more pathogenic properties and gut dysbiosis, while others are less so [63].
- **Gut-Brain Axis Research:** Emerging studies are exploring the connection between *Blastocystis* colonization and the gut-brain axis, suggesting potential links to behavioral disorders like anxiety and depression in animal models, or a protective role in chronic stress depending on the subtype [64].
- **"Pathobiont" Concept:** Researchers are considering whether *Blastocystis* might be a "pathobiont," a normal resident of the microbiome that can cause disease only under specific host or environmental conditions [65].

Therapeutic and Research Directions

- **Alternative Treatments:** Beyond conventional antibiotics like metronidazole (which has variable efficacy and resistance issues), interest is growing in alternative therapies such as probiotics (e.g., *Saccharomyces boulardii*), medicinal plant extracts, and dietary interventions [66].
- **"One Health" Approach:** A major ongoing initiative, the COST Action CA21105 "Blastocystis under One Health," emphasizes a collaborative, multidisciplinary approach integrating human, veterinary, and environmental health to better understand transmission dynamics and public health implications[67].
- **Improved In Vitro and In Vivo Models:** Efforts are underway to expand the range of axenic (pure) cultures for different STs and develop better animal models (e.g., gut-on-a-chip devices) to facilitate in-depth studies on pathogenicity and host interactions, which has been a major historical challenge [68].

Moreover, innovative research explored drug discovery approaches against Blastocystis by leveraging methodologies used against pathogenic free-living amoebae (FLA). By applying anti-amoebic drug discovery techniques, this study targeted the amoeboid forms of Blastocystis, which may resemble those of FLAs [69]. Despite many studies investigating the association between Blastocystis presence and its clinical significance, the latter remains a topic of debate. Preliminary data from an ongoing project examining the associations of gallbladder removal surgery and the presence of Blastocystis STs, as well as how this influences microbiome composition and metabolite profiles, showed that several patients who were non-carriers before surgery became colonised post-surgery [70]. Addressing the ongoing debate on its pathogenic potential, a scoping review, adhering to PRISMA guidelines, aims to clarify the associations between *Blastocystis* infection and gastrointestinal health outcomes. This initiative aspires to fill knowledge gaps, potentially guiding future research in compliance with the COST action objectives [71].

Pathogenesis

The following Blastocystis taxonomic classification is used at present: Kingdom: Sar; Phylum: Stramenopiles; Class: Bigyra; Order: Opalinata; Family: Blastocystidae; Genus: Blastocystis [5,6], while the classification species-level is still unsolved. Historically, the name of species have been given depending on the hosts of which the parasite has been isolated for example

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(*Blastocystis hominis* or *Blastocystis. ratti*). Studies demonstrated that the specificity of hosts and pathogenic potentials are related to differences in the small subunit ribosomal RNA (SSU rRNA) sequence [72,73]. Among different humans and animals, at least 42 *Blastocystis sp.* Subtypes were detected by the molecular SSU rRNA gene analysis [9], with the suspicion of some subtypes to be pathogenic [10]. Until now, 16 subtypes were detected, with the most commonly association of ST1–ST4 with infections [74,75]. Considering the classification of unsettled species, the correct nomenclature is *Blastocystis sp.,* with determining the subtypes according to the analysis of molecular SSU rDNA or SSU rRNA [76].

Different morphological forms of Blastocystis sp. are exhibited with variable structures and sizes which can transit between forms in responses to the environmental factor. Such forms involve include granular, vacuolar, non-vacuolar, multi-vacuolar, amoeboid as well as cystic form [77]. Transmissions occur through the fecal–oral routes, with the cysts being as the infectious stages. In the gastrointestinal tract of the host, the cysts undergo excystations following ingestion, and release vacuolar forms that can undergo binary fissions. After that, such forms become encysted in the gut lumen to produce new cysts which excrete in the stool, and complete the transmission cycles [78].

Infections of humans with *Blastocystis sp.* mostly occur due to ingestions of cysts that exist in contaminated foods and water i.e. (inadequately washed fruit and vegetable). Transmission may also occur directly via animal reservoir contact such as livestocks (pig, goat, sheep, cattles) as well as birds [79]. Many risk factors affect the infection prevalence including sanitation infrastructures, hygiene practice, age, overall health, nutritional state, and lifestyle's habits, e.g. water consumption practices and hand's hygiene [80].

Approximately one billion individuals are estimated in the world to be infected with Blastocystosis, with a prevalence rate range from 1.5 to 10% in developed countries and 30 to 50% in developing countries [81]. Immunocompetent people are usually without symptoms or show mild and non-specific symptoms e.g. bloatings and bowel cramps [82]. Nevertheless, immunocompromised persons, such as patients having cancer HIV/AIDS, organ transplantation, and patients taking immunosuppressive drugs or haemodialysis, are subject to infection in particular. Among high and middle income countries, it is estimated that the prevalence rate is 10% [83,84]. Because of progressive immune dysfunction, these patients with Blastocystosis can develop serious diarrhea [85]. It is suggested that animal models with impaired immunity exacerbate disease severity, as confirmed by widespread intestinal involvements and increased pro-inflammatory antibody and cytokine production in comparison with the immunocompetent people [86].

Symptoms of blastocystis sp. infection may include abdominal pains, chronic diarrhea, nausea, vomiting, anorexia, weight losses as well as general weaknesses. Moreover, non-gastrointestinal symptom like rashes, pruritus in addition to joint pains were observed [87]. In immunosuppressed patients or patients with comorbidities , Blastocystosis may be a life threatening disease [88].

In general, diagnosis depends upon direct microscopic fecal smear examinations [89]. The vacular forms are most commonly identified, while other morphologic forms are more challenging to detect and might be confused with organisms, leucocytes, lipid droplet and other faecal constituents [90]. Serological techniques are inadequately used, since their diagnostic values are still to be investigated. Culture methods are time-consuming and need special growth conditions which are difficultly maintained, although they provide high sensitivity [91]. Based on such limitations, molecular diagnostic methods, especially polymerase chain reactions are highly preferred. PCR provides high sensitivity and enables subtype's differentiations [92,93,94]. It was indicated by some studies that PCR is favored more than the traditional identification procedures to confirm Blastocystosis [95]. Moreover, PCR method showed

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higher specificity, sensitivity and predictive results in comparison with microscopic and culturing methods [96,97,98].

Blastocystis sp. role in the health of human's gastrointestinal health is still a subject of continuing scientific investigations [99,100]. Researches are investigating its potential contribution to colorectal cancer pathogenesis, irritable bowel's syndrome (IBS) as well as autoimmune disease like Hashimoto thyroiditis and ulcerative colitis [101,102,103].

4. CONCLUSION

The diagnosis of Blastocystis (formerly Blastocystis hominis) relies on identifying the organism in stool samples. Recent advances highlight the use of more sensitive and specific methods: Microscopy: Traditional examination of permanently stained stool smears (e.g., trichrome or iron hematoxylin) remains a standard method in clinical labs, with trichrome staining often considered the most practical for routine use. The presence of numerous organisms (e.g., >5 per high-power field) in symptomatic patients suggests pathogenicity.

Molecular Methods: Real-time polymerase chain reactions (PCR) is the major sensitive and specific diagnostic available technique, particularly useful for detecting low parasite loads and identifying specific Blastocystis subtypes (genotypes). Subtyping is an important research advance as some subtypes are more associated with symptoms and drug resistance than others.

Culture: In vitro culture techniques are also more sensitive than direct microscopy and can be used in laboratories with limited molecular resources.

Treatment Advances and Approach

The key consensus in treatment is that asymptomatic individuals generally do not require treatment. For symptomatic patients (e.g., persistent diarrhea, abdominal pain, bloating, nausea, or associated skin rashes) where no other pathogen is identified, treatment may be warranted.

Key treatment points:

First-line therapy: Metronidazole is the most frequently prescribed first-line drug, typically at a dosage of 500-750 mg three times daily for 7-10 days.

Variable Efficacy and Resistance: A significant advance is the recognition of wide variability in metronidazole efficacy (0% to 100% eradication rates) and the existence of drug-resistant Blastocystis subtypes. This has led to the exploration of alternative options.

Alternative and Adjunct Therapies: For patients who fail initial treatment, alternative options include: Trimethoprim-sulfamethoxazole (TMP-SMX).

Nitazoxanide, a broad-spectrum antiparasitic agent, which has shown good efficacy in some studies. Paromomycin, especially in cases of treatment failure or for cutaneous manifestations. The probiotic yeast Saccharomyces boulardii has shown potential beneficial effects in clinical and parasitological cure rates, in some cases comparable to metronidazole.

Combination Therapy: For refractory cases, combination regimens (e.g., metronidazole and paromomycin, or nitazoxanide, furazolidone, and secnidazole) may be considered, although some drugs may not be widely available.

Monitoring: Clinical follow-up with repeat stool examinations 2-4 weeks after treatment is recommended to confirm eradication, especially if symptoms persist. Overall, the field has advanced from viewing Blastocystis as a uniform commensal to recognizing its species diversity, variable pathogenicity based on subtype, and the need for individualized treatment strategies when indicated.

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5. Competing interest disclaimer:

Authors declare that they have no known competing financial or non financial interest or personal relationships that could have appeared to influence the work reported in this paper

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