**Blastocystis**, an unrecognized parasite: an overview of pathogenesis and diagnosis

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**Abstract:** *Blastocystis* sp. is among the few enteric parasites with a prevalence that often exceeds 5% in the general population of industrialized countries and can reach 30–60% in developing countries. This parasite is frequently found in people who are immunocompromised (patients with human immunodeficiency virus/acquired immunodeficiency syndrome or cancer) and a higher risk of *Blastocystis* sp. infection has been found in people with close animal contact. Such prevalence in the human population and the zoonotic potential naturally raise questions about the impact of these parasites on public health and has increased interest in this area. Recent *in vitro* and *in vivo* studies have shed new light on the pathogenic power of this parasite, suggesting that *Blastocystis* sp. infection is associated with a variety of gastrointestinal disorders, may play a significant role in irritable bowel syndrome, and may be linked with cutaneous lesions (urticaria). Despite recent significant advances in the knowledge of the extensive genetic diversity of this species, the identification of extracellular proteases as virulence factors and the publication of one isolate genome, many aspects of the biology of *Blastocystis* sp. remain poorly investigated. In this review, we investigate several biological aspects of *Blastocystis* sp. (diversity and epidemiology, diagnosis tools and pathophysiology). These data pave the way for the following challenges concerning *Blastocystis* sp. research: deciphering key biological mechanisms and pathways of this parasite and clarification of its clinical impact in humans.

**Keywords:** Blastocystis, pathogenesis, diagnosis, subtypes, gut

*Blastocystis* sp. is an anaerobic intestinal parasite of humans and a wide range of animals [Stenzel and Boreham, 1996; Tan, 2004, 2008]. This parasite belongs to the stramenopiles, a complex and heterogeneous evolutionary assemblage of heterotrophic and photosynthetic protozoa [Silberman et al. 1996; Arisue et al. 2002; Rübsberg et al. 2009]. Interestingly, *Blastocystis* sp. is the only stramenopiles known to cause infection in humans. Four major morphological forms of *Blastocystis* sp. were described in stools or *in vitro* cultures: vacuolar (Figure 1), granular, amoeboid and cyst forms [Stenzel and Boreham, 1996; Tan, 2008; Suresh et al. 2009]. The two former forms are the most easily recognizable and frequently observed in laboratory culture and stool samples. Although rarely reported, the irregular amoeboid form was postulated to play a role in pathogenesis [Tan et al. 2006, Katsarou-Katsari et al. 2008 but this hypothesis was contradicted [Souppart et al. 2009]. Experimental infectivity studies in animals with the cyst form demonstrated that the water-resistant and environmentally resistant infective cysts represented the transmissible stage of the parasite [Suresh et al. 1993, 2005; Moe et al. 1996; Yoshikawa et al. 2004]. Taking into account these observations and those of *in vitro* encystations studies [Suresh et al. 1993; Villar et al. 1998; Chen et al. 1999], a life cycle for *Blastocystis* sp. was proposed with the cyst as the infectious stage [Tan, 2008]. Upon ingestion of cysts, the parasite undergoes encystation in the large intestine and develops into vacuolar forms. These vacuolar forms divide by binary fission and may develop into amoeboid or granular forms. Then, encystation may occur while crossing along the colon before cyst excretion in the faeces [Tan, 2008]. Therefore, *Blastocystis* sp. lives in oxygen-poor environments and is...
characterized by the presence of some double-membrane surrounded organelles called mitochondrion-like organelles (MLOs) (Figure 1) [Nasirudeen and Tan, 2004]. Sequencing of complete circular DNA in the MLO of these parasites by different authors [Perez-Brocal and Clark, 2008; Stechmann et al. 2008; Wawrzyniak et al. 2008] showed that the cellular compartments have the metabolic properties of both aerobic and anaerobic mitochondria. The nuclear genome of Blastocystis sp. was recently sequenced and revealed a compact nature and an intriguing architecture [Denoeud et al. 2011]. This genome has also provided clues to decipher the genetic diversity and pathogenesis of this parasite.

Summary of diagnosis tools

The most common approaches for the detection of Blastocystis sp. [Stenzel and Boreham, 1996; Stensvold et al. 2007a; Tan, 2008] consist of direct smear examination by light microscopic or xenic in vitro culture. However, given the occurrence of different forms of Blastocystis sp. (especially the hardly recognizable cystic form), deterioration caused by environmental conditions or drug treatment and the fact that Blastocystis sp. can be confused with other microorganisms, this method seems to have largely underestimated this parasite in the context of enteric parasite diagnosis. Moreover, culturing this parasite is time-consuming and can bias subsequent genotyping due to the different ability of isolates to grow in selective medium [Roberts et al. 2011]. Therefore, to overcome these limitations, several molecular polymerase chain reaction (PCR)-based diagnostic approaches using faeces directly or after culture of faecal specimens have been described [Santin et al. 2011; Abe et al. 2003a; 2003b; Yoshikawa et al. 2004; Scicluna et al. 2006; Stensvold et al. 2006; Roberts et al. 2011]. Studies comparing the relative performances of these various diagnostic methods [Suresh and Smith, 2004; Parkar et al. 2007; Stensvold et al. 2007a] showed that the PCR approach was as sensitive as the culture approach. More recently, Poirier and colleagues reported a highly sensitive real-time quantitative PCR (qPCR) assay developed to detect Blastocystis sp. in stool samples [Poirier et al. 2011]. This assay targets a region of the small subunit rRNA gene (SSU-rDNA) and allowed subtyping of isolates by direct sequencing of qPCR products. Moreover, Stensvold and colleagues developed a qPCR on stool samples using the SSU-rDNA marker, including an internal process control enabling the evaluation of potential PCR inhibitors [Stensvold et al. 2012]. This approach had the advantage of increasing the specificity and avoiding the amplification of false positives. Therefore, currently, SSU-rDNA genotyping is the method of choice for diagnosis [Poirier et al. 2011; Stensvold et al. 2012].
**Blastocystis sp., a highly prevalent and divergent parasite**

Numerous epidemiological surveys carried out in different countries identify *Blastocystis* sp. as the most common eukaryotic parasite reported in human faecal samples [Tan, 2008]. Overall the prevalence of *Blastocystis* sp. is higher than those of other intestinal protozoan parasites such as *Giardia*, *Entamoeba* and *Cryptosporidium*, as observed in France [The ANOFEL Cryptosporidium National Network, 2010] and the United States [Boorom et al. 2008]. An increasing trend in identification of *Blastocystis* sp. suggests that it is an emerging parasite with a worldwide distribution [WHO, 2008]. Prevalence varies widely from country to country and within communities of the same country [Tan, 2008; Souppart et al. 2009; Alfellani et al. 2013]. Developing countries have a higher prevalence of the parasite than industrialized countries. This difference can be explained by poor hygiene practices, close animal contact and consumption of contaminated food or water [Li et al. 2007; Leelayoova et al. 2008; Eroglu and Koltas, 2010; Baldursson and Karanis, 2011; Ithoi et al. 2011; Lee et al. 2012; Nagel et al. 2012] since the faecal–oral route is considered to be the main mode of transmission of this parasite [Yoshikawa et al. 2004]. Prevalence is low in developed countries such as Japan (0.5–1%) [Hirata et al. 2007] and Singapore (3.3%) [Wong et al. 2008] and high in developing nations including Brazil (40.9%) [Aguiar et al. 2007], Egypt (33.3%) [Rayan et al. 2007] and Indonesia (60%) [Pegelew et al. 1997]. In some countries, the prevalence can be rather variable and ranges from 1.9% to 32.6% in China [Li et al. 2007] and from 0% to 45.2% in Thailand [Saksirisampant et al. 2003, 2006], depending on the subpopulation studied. Such variations within the same country could reflect true differences between communities or the use of different diagnostic approaches [Stensvold, 2013].

*Blastocystis* sp. isolates from humans and other animals have been reported to be morphologically indistinguishable. However, extensive genetic variation among numerous *Blastocystis* sp. isolates from both humans and animals was mainly observed by PCR restriction fragment length polymorphism [Hoegers et al. 2000; Kaneda et al. 2001; Abe et al. 2003b; Rivera and Tan, 2005] and PCR using sequenced-tagged site primers [Yoshikawa et al. 2004; Yan et al. 2006; Li et al. 2007; Yoshikawa et al. 2009]. This considerable genetic divergence among isolates was subsequently confirmed by molecular phylogenies, mainly inferred from SSU-rDNA sequences [Arisue et al. 2003; Abe, 2004; Noël et al. 2005; Scicluna et al. 2006; Jones et al. 2008; Ozurt et al. 2008; Souppart et al. 2009, 2010; Stensvold et al. 2009; Parkar et al. 2010; Whipps et al. 2010]. From these molecular analyses, a consensus on *Blastocystis* sp. terminology was proposed in an international collaborative project [Stensvold et al. 2007b]. In this new classification, all isolates should be designated *Blastocystis* sp. and assigned to one of the described subtypes (STs). Each of the STs exhibiting sufficient genetic diversity should be classified as separate species. Thereafter, a new ST was identified from primates and artiodactyls and designated as *Blastocystis* sp. ST10 [Stensvold et al. 2009]. More recently, seven additional STs (ST11–17) were identified from zoo animals [Parkar et al. 2010; Fayer et al. 2012; Alfellani et al. 2013; Roberts et al. 2013].

Most of the samples included in published epidemiological surveys represented simple infections. Mixed infections (i.e. infections by at least two different STs) probably result from multiple sources of infection. The prevalence of these infections is similar in different countries and roughly comprised between 2.6% and 14.3% [Yan et al. 2006; Li et al. 2007; Dogruman-Al et al. 2008; Souppart et al. 2009; Meloni et al. 2012]. However, the true distribution of mixed infections remains difficult to ascertain in a particular individual and is likely underestimated as this depends on the method employed for subtyping [Meloni et al. 2012]. In almost all the studies reported so far, including those in Europe [Souppart et al. 2009; Meloni et al. 2011; Forsell et al. 2012; Alfellani et al. 2013], Africa [Souppart et al. 2010; Alfellani et al. 2013], Oceania [Roberts et al. 2013], Asia [Jantermtor et al. 2013] and the Middle East [Moosavi et al. 2012], a large majority of human infections with *Blastocystis* sp. were attributable to ST3 isolates. Only a few exceptions showed a higher prevalence of ST4 in Spain [Dominguez-Marquez et al. 2009], Denmark [Stensvold et al. 2011] and in a region of France [Poirier et al. 2011], and of ST1 in Thailand [Thathaisong et al. 2013]. Collectively, these studies suggest that the dominant ST3 was the only ST of human origin as was first proposed by Noël and colleagues [Noël et al. 2005], even if it can also be found in some...
animals. Consequently, the predominance of this ST might be mainly explained by large-scale human to human transmission [Yoshikawa et al. 2000]. Proportions of ST1 to ST4 differ between locations. ST6 and ST7 are common in Asia but rarely observed in European countries. ST5, ST8 and ST9 are found episodically in humans [Yan et al. 2007; Tan, 2008; Stensvold et al. 2009; Tan et al. 2010; Moosavi et al. 2012].

Comparison of SSU-rDNA gene sequences, cross-transmission experiments and respective prevalence of different STs in the human population indicate that almost all of the known STs of supposed animal origin are likely zoonotic and able to infect human (Figure 2) [Noël et al. 2005; Parkar et al. 2007, 2010; Yan et al. 2007; Yoshikawa et al. 2009]. Therefore, a higher risk of Blastocystis sp. infection was found in people with close animal contact, including zoo keepers [Parkar et al. 2010] and abattoir workers [Rajah Salim et al. 1999; Parkar et al. 2010], indicating that animals may represent a significant zoonotic source of this parasite for humans.

**Insights into Blastocystis sp. pathogenesis**

The pathogenic status of Blastocystis sp. was widely debated in the literature to determine whether this microorganism was a truly pathogenic or commensal organism [Stenzel and Boreham, 1996; Boorom et al. 2008; Tan, 2008; Tan et al. 2010], although an increasingly number of recent studies cited Blastocystis sp. as an emerging pathogen [Tan, 2008, Tan et al. 2010; Poirier et al. 2012; Scanlan, 2012]. This is mainly due to the fact that Blastocystis sp. can be found in both symptomatic and asymptomatic patients [Dogruman-Al et al. 2008; Eroglu et al. 2009; Souppart et al. 2009]. However, recent in vitro and in vivo studies show that Blastocystis sp. infection is associated with a variety of gastrointestinal disorders (called blastocystosis), especially in irritable bowel syndrome (IBS) and cutaneous lesions.

*Blastocystis* sp. is associated with various clinical symptoms in humans

In humans, blastocystosis is mainly characterized by nonspecific gastrointestinal symptoms, like
diarrhoea, abdominal pain, flatulence, nausea, vomiting, constipation, weight loss or fatigue [Stenzel and Boreham, 1996; Boorum et al. 2008; Tan, 2008; Tan et al. 2010]. The severity of these diseases is variable and ranges from acute to chronic infections [Tan, 2008]. A hypothesis to explain differences in the disease caused by Blastocystis sp. is its genetic diversity [Hussein et al. 2008; Tan, 2008; Tan et al. 2010; Scanlan, 2012], although no association was detected between symptoms and Blastocystis subtypes in several studies [Ozyurt et al. 2008; Dogruman-Al et al. 2009; Souppart et al. 2009; Jantermtr et al. 2013]. However, ST4 isolates are more common in symptomatic patients in Sweden, Denmark and Spain [Dominguez-Marquez et al. 2009; Stensvold et al. 2011; Forsell et al. 2012], arguing for an important role of this subtype that needs to be investigated further.

In addition to aspecific gastrointestinal symptoms, studies associated the parasite with cutaneous disorders and chronic or acute urticaria [Vogelberg et al. 2010; Hameed et al. 2011; Zuel-Fakkar et al. 2011; Verma and Delfanian, 2013]. These diseases were correlated with the presence of Blastocystis sp. belonging to the ST2 [Vogelberg et al. 2010] or ST3 [Zuel-Fakkar et al. 2011] in the patient stools. An association was also found between urticaria and amoeboid forms of a ST3 isolate [Katsarou-Katsari et al. 2008]. It was suggested that the amoeboid form adheres efficiently to the intestinal epithelium, affecting gut immune homeostasis and causing an inflammatory response against the parasite that led to urticaria [Valsecchi et al. 2004].

Blastocystis sp. is also suspected to be involved in IBS [Tan et al. 2010; Poirier et al. 2012; Scanlan, 2012]. Indeed several studies reported a higher incidence of the parasite in patients with IBS compared with healthy populations [Poirier et al. 2012]. IBS is a common gastrointestinal disorder characterized by abdnominal pain and discomfort associated with changes in bowel habits [Longstreth et al. 2006]. Studies on the IBS population showed a higher prevalence of ST1 and ST3 isolates of Blastocystis sp. [Yakoob et al. 2010; Fouad et al. 2011; Jimenez-Gonzalez et al. 2012]. However, these studies did not conclude that Blastocystis sp. was the sole etiologic agent. Moreover, a recent study demonstrated that even though Blastocystis sp. was more frequent in symptomatic patients with IBS, the differences compared with controls were not significant [Cekin et al. 2012]. The presence of Blastocystis sp. in symptomatic patients can also indicate that this parasite could be involved with other factors in this disease pathophysiology [Poirier et al. 2012]. It is possible that the alteration of the intestinal environment, provoked by pathogens (bacteria), genetic or environmental factors promotes its development. Furthermore, studies showing the concomitant eradication of Blastocystis sp. with the disappearance of symptoms in patients with IBS are needed to clarify the role of this parasite.

High-risk populations
As in many parasitic infections, some populations are more susceptible to Blastocystis sp. infection. Therefore, this parasite is frequently found in immunocompromised individuals such as those with human immunodeficiency virus/acquired immunodeficiency syndrome or cancer [Kurniawan et al. 2009; Tan et al. 2010]. Children from developing countries and those who are immunocompromised are also more susceptible [Calik et al. 2011; Canete et al. 2012; Daryani et al. 2012]. The socioeconomic status, the quality of drinking water, the consumption of contaminated food and the personal hygiene habits are the major risks explaining contamination in children [Abdulsalam et al. 2012; Canete et al. 2012]. A higher risk of Blastocystis sp. infection was also found in people who are in close contact with animals, which increases exposure to the parasite [Yoshikawa et al. 2009; Parkar et al. 2010], reinforcing the zoonotic nature of Blastocystis sp.

Pathophysiology of blastocystosis
One of the major obstacles to the study of the pathogenesis of Blastocystis sp. is the lack of animal models to test Koch’s postulate. However, a variety of experimental infections involving different animals have been described, including rats, mice, guinea pigs or chickens [Tan, 2008; Tan et al. 2010]. From these, it was deduced that laboratory mice are not suitable as animal models. Infections were generally self-limiting, although some mice showed weight loss and lethargy. However, histological examination of the cecum and colon revealed intense inflammatory cell infiltration, oedematous lamina propria and mucosal sloughing [Moe et al. 1997]. These authors also showed that there was an age-related susceptibility to Blastocystis sp. in mice. Indeed, juvenile BALB/c mice were more susceptible
than adult mice and 8-week-old adult BALB/c mice were totally resistant to Blastocystis sp.

Studies on rat models suggest that this species is more suitable for developing an animal model [Tan, 2008]. Ten to a hundred cysts were able to establish an infection via intracecal or oral inoculations in 3-week-old Wistar rats and all contents from the cecum and large intestine were positive for Blastocystis sp. infection [Yoshikawa et al., 2004]. Hussein and colleagues tested the infectivity of human ST1–4 isolates obtained from both asymptomatic and symptomatic patients in 4-week-old male Wistar rats orally inoculated with 4-day-old cultures of Blastocystis sp. negative for Cryptosporidium, Cyclospora, Isospora, Microsporidium and common bacterial pathogens [Hussein et al., 2008]. Interestingly, the moderate and severe degrees of pathological changes observed 6 weeks post infection were found only with symptomatic isolates while mild changes were found only with asymptomatic isolates. Interestingly, ST1 symptomatic isolates induced 25% mortality in rats, indicating its pathogenesis. In parallel, the authors described intense inflammatory reaction and sloughing mucosa, oedema and precancerous polyps in cecum and proximal colon tissues in symptomatic infected rats. It was suggested that the inflammation induced by Blastocystis sp. had the ability to alter tight junctions between the intestinal epithelial cells and the intestinal content, which led to disturbances of the barrier function and permeability [Hussein et al. 2008]. However, the possibility that these pathologies could be of bacterial or viral origin was not excluded.

Some in vitro studies were conducted to investigate the mechanisms of physiopathology by studying the cytopathic effects of Blastocystis sp. on mammalian cell cultures. A first study [Long et al. 2001] showed that 24 h incubation with Blastocystis sp. ST1 cells or culture filtrates induced the production of proinflammatory cytokines interleukin (IL)-8 and granulocyte–macrophage colony-stimulating factor, suggesting that the parasite was able to modulate the host immune response (Figure 3). The induction of IL-8 production from human colonic epithelial cells (HT84) was demonstrated to be activated by proteases released by Blastocystis sp. that may participate in the attack of intestinal epithelium with other hydrolases and may cause the increase in paracellular permeability that is observed in several digestive pathologies such as irritable bowel syndrome (IBS). Blastocystis sp. is able to induce physiological disturbances linked to IBS: host cell apoptosis, the modulation of host immune response and a microinflammation. Some as yet uncharacterized secondary metabolites produced by the polyketide synthase or nonribosomal peptide synthases could participate in host intestinal symptoms by inducing changes in the host microbiota, another feature of IBS. Finally, drug-resistant isolates of the parasite could be explained by the presence of multidrug resistance proteins that could eject active drugs. GM-CSF, granulocyte–macrophage colony-stimulating factor; Ig, immunoglobulin; IL, interleukin; INF, interferon; TNF, tumour necrosis factor.

Figure 3. Mechanisms of blastocystosis physiopathology. Blastocystis sp. may release cysteine protease that may participate in the attack of intestinal epithelium with other hydrolases and may cause the increase in paracellular permeability that is observed in several digestive pathologies such as irritable bowel syndrome (IBS). Blastocystis sp. is able to induce physiological disturbances linked to IBS: host cell apoptosis, the modulation of host immune response and a microinflammation. Some as yet uncharacterized secondary metabolites produced by the polyketide synthase or nonribosomal peptide synthases could participate in host intestinal symptoms by inducing changes in the host microbiota, another feature of IBS. Finally, drug-resistant isolates of the parasite could be explained by the presence of multidrug resistance proteins that could eject active drugs. GM-CSF, granulocyte–macrophage colony-stimulating factor; Ig, immunoglobulin; IL, interleukin; INF, interferon; TNF, tumour necrosis factor.
by cysteine proteases from Blastocystis sp. ST4 in a nuclear factor kB dependent manner [Puthia et al. 2008]. The same authors also showed that coinoculation of intestinal epithelial cells of rat intestinal IEC6 with either Blastocystis sp. ST4 isolates or parasite lysates induced the apoptosis of host cells [Puthia et al. 2006] in a contact-independent manner. A decrease in transepithelial resistance and an increase in epithelial permeability were also observed and could be explained by the rearrangement of actin filaments [Tan et al. 2010; Mirza et al. 2012]. These data suggest that Blastocystis sp. is able to disturb host gut homeostasis (Figure 3). As observed in other parasitic protozoa [Sajid and McKerrow, 2002], cysteine proteases of Blastocystis sp. should be involved in parasite survival in vivo and represent virulence factors [Tan et al. 2010; Scanlan, 2012]. Variations in cysteine protease activity were observed between ST4 and ST7 isolates, which may be attributable to differences in virulence [Mirza and Tan, 2009]. Protease activities were identified in Blastocystis sp. secretory products and were able to cleave human secretory immunoglobulin A, the prevalent immunoglobulin defence at the mucosal surface [Puthia et al. 2005]. Another study revealed that cysteine proteases increased permeability of human epithelium by reorganization of the tight junction complex and modulation of the rho associated kinase/phosphorylation of myosin light chain pathway [Mirza et al. 2012]. The sequence of the Blastocystis sp. ST7 complete genome provides molecular candidates that could be involved in pathogenesis [Denoeud et al. 2011]. Twenty-two proteases were predicted to be secreted [Denoeud et al. 2011] and could therefore play a role at the host–parasite interface. Among them, 20 cysteine proteases, 1 serine protease and 1 aspartic protease were characterized and 2 cysteine proteases were experimentally identified and characterized in the secretory products by mass spectrometry analysis [Wawrzyniak et al. 2012]. These secreted proteases are serious candidates to explain gut function disruption observed in intestinal pathologies [Poirier et al. 2012]. Apart from proteases, hydrolases and protease inhibitors were predicted to be secreted and could participate in the blastocystosis physiopathology. Moreover, genes coding a polyketide synthase and nonribosomal peptide synthases were identified [Denoeud et al. 2011]. These enzymes may produce molecules with interesting pharmacological activities like antibiotics and toxins and may be implicated in dysbiosis (Figure 3). The next challenge is to understand the role of Blastocystis sp. in gut dysfunctions [Poirier et al. 2012].

Treatment of blastocystosis

This treatment is usually considered if diarrhoea is persistent and no other pathogen apart from Blastocystis sp. is identified in faecal specimens [Coyle et al. 2012]. In this case, metronidazole is considered as the first-line therapy for Blastocystis sp. infection [Nigro et al. 2003; Cassano et al. 2005; Moghaddam et al. 2005; Stensvold et al. 2010]. In a first evaluation of this drug efficacy, Nigro and colleagues showed that immunocompetent individuals with Blastocystis sp. infection as the only evident cause of diarrhoea responded to metronidazole treatment and consequently they suggested that the parasite induced intestinal disease [Nigro et al. 2003]. However, there were accumulating reports of treatment failure, particularly in patients with severe Blastocystis sp. infections [Haresh et al. 1999; Moghaddam et al. 2005; Stensvold et al. 2008, 2010], suggesting the existence of extensive variations in drug susceptibility [Mirza et al. 2011]. Accordingly, some genes coding for multidrug resistance pump proteins (ATP-binding cassette transporters) were identified in the Blastocystis sp. ST7 genome (Figure 3) [Denoeud et al. 2011]. Several standard antimicrobials (cotrimoxazole, ornidazole, nitazoxanide, paromomycin, chloroquine, trimethoprim-sulfamethoxazole, iodoquinol, tinidazole, emetine, pentamidine, iodochlorhydroxyquin and furazolidone) may be considered as second-choice drugs [Ok et al. 1999; Cimerman et al. 2003; Diaz et al. 2003; Rossignol et al. 2005; Stensvold et al. 2008, 2011; Coyle et al. 2012]. Even if some of these drugs were shown to be globally effective against Blastocystis sp., treatment failures were also largely reported [Haresh et al. 1999; Nigro et al. 2003; Mirza et al. 2011].

Conclusion

Blastocystis sp. was included in the Water Sanitation and Health programmes of the World Health Organization [WHO, 2008]. Increasing interest of the scientific and medical community in Blastocystis sp. was coupled with new data about epidemiology, pathogeny and more recently the first whole genome of Blastocystis ST7. Accumulating in vivo, in vitro and in silico data assessed the importance of Blastocystis sp. in human health, with probably the association with major host and environmental factors to be determined. To answer most of
the crucial questions regarding Blastocystis sp., we need to develop and standardize axenization of new STs and diagnosis tools, transfection and animal models, microarrays and genotyping markers. Sequencing of genomes from different STs and comparative genomic projects are in progress and will be useful to identify specific virulence factors. Multicentre studies are also required to further our comprehension of the clinical implications of Blastocystis sp. in IBS and other pathologies. To conclude, the interaction of Blastocystis sp. with gut microbiota needs to be studied because of the increasing interest in microbiota disturbances in the genesis of various gastrointestinal dysfunctions.

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References


