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Blastocystis sp. Subtypes Colonisation and their Association with Clinical Diseases: A Systematic Review

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Blastocystis sp. has been considered as an opportunistic intestinal parasite particularly in immunocompromised patient. Recent findings of the predominance of *Blastocystis* subtypes infection in the clinical disease with its interaction with gut microbiota in the hosts will be discussed accordingly. A total of 57 eligible studies, published from 2010 to October 2020 from a broad search in electronic databases were accessed. The studies showed that bloating, abdominal pain and diarrhoea were among the common symptoms in *Blastocystis* sp. infection in the immunocompromised patients. However, asymptomatic and healthy individuals were also infected by the *Blastocystis* sp. with higher prevalence among healthy individuals. Specifically, *Blastocystis* sp. ST3 were most frequently discovered among immunocompromised patients (IBS, cancer, transplant, HIV/AIDS, dengue) followed by ST1 and ST2. Despite most studies suggesting that *Blastocystis* sp. promote a healthy gut, a few studies had suggested otherwise. *Blastocystis* sp. colonisation may modify the gut microbiota with the reduction of beneficial bacteria phyla such as Firmicutes and Bacteroides. Although there was a positive association between *Blastocystis* sp. subtypes and clinical diseases, more studies are needed. To understand the pathogenicity of *Blastocystis* sp., their interactions with the gut microbiota communities in humans are properly discussed.

Keywords: *Blastocystis* sp.; subtypes; immunocompromised; gastrointestinal symptoms; clinical disease; gut microbiota

I. INTRODUCTION

Blastocystis sp. or previously known as *Blastocystis hominis* is one of the most frequent protozoan parasites discovered in human intestines with global distribution, including developing countries (Yersal *et al.*, 2016). It is believed that the parasite was under reported due to its poorly investigated pathogenicity, clinical relevance and therapeutic need, particularly in immunocompromised individuals (Wawrzyniak *et. al.*, 2013; Cristanziano *et al.*, 2019). At an early proposed by Alexeieff (1911), *Blastocystis* sp. was regarded as a different organism which has been called as *Blastocystis enterocola*. Then, it was renamed to *Blastocystis hominis* by Brumpt in 1912 (Zierdt, 1991; Silberman *et al.*, 1996). Later, the phylogenetic studies suggested "*Blastocystis* sp." as a term that we use nowadays due to genetic diversity exhibited by the members within the genus (Stensvold *et al.*, 2007). The organism has been reclassified into a separate group, stramenophile, a heterotrophic-photosynthetic protist and the only organism in stramenophile that could lead to human infection (Roberts *et al.*, 2014). To date, approximately 1 billion

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people worldwide have been infected with *Blastocystis* sp. with asymptomatic presentation. Its incidence varies from country to country depending on hygiene and health conditions, proximity to livestock, immune status of hosts, various geographical areas, age of hosts and also contamination of food and water (Deng *et al.*, 2019). In industrialised countries, the rates of *Blastocystis* sp. infection are exceeding 5% and reaching 30-60% in developing countries. It is suggested that faecal-oral route is the main mode of transmission with 22-56% of human infection in European countries and 37-100% in Asia and Africa (Popruk *et. al.*, 2015; Forsell *et al.*, 2017).

Currently, there are 26 *Blastocystis* sp. subtypes (STs) reported with remarkable genetic diversity that are isolated based on the comparison of the small-subunit rRNA gene (SSU rDNA) orders. It has been represented by 22 of these genetically distinct subtypes (ST1-ST17, ST21, and ST23-ST26) with exceptional of subtypes (ST18, ST19, ST20 and ST22) which suggested to be invalid due to their appearance of being potentially chimeric (Maloney *et. al.*, 2019; Stensvold & Clark, 2020; Maloney & Santin, 2021). To date, 12 subtypes (STs) have been identified in humans (ST1-ST10, ST12, and ST14); around 90 – 95% of human infections are caused by one of the ST1-ST4 subtypes, with ST3 being the most prominent (Clark *et. al.*, 2013; Khaled *et. al.*, 2020; Zanetti *et al.*, 2020). Meanwhile, with the exception of ST9, all of the subtypes seen in humans have been detected in animals including non-human primates, birds and mammals (Valenca *et. al.*, 2019; Zanetti *et al.*, 2020). It has been proposed that different subtypes are linked to *Blastocystis* sp. pathogenicity (Ajjampur & Tan, 2016). However, the findings indicated that not all strains of a given subtype are pathogenic. *Blastocystis* sp. pathogenicity is strongly dependent on the organism's pan genome, which encodes for virulence elements and toxins. Thus, this demonstrates that the subtype is not the only factor associated with pathogenicity of this parasite (Roberts *et. al.*, 2014; Tito *et al.*, 2019). Interestingly, *Blastocystis* sp. is not only regarded as a pathogen, but is also as a potential commensal as distinct STs are present in asymptomatic hosts or as a biomarker of gut functionality due to a correlation with specific intestinal microbial populations (Scanlan *et al.*, 2013). Gut microbiota are being characterised as the huge diversity, the resilience, the stability and the symbiotic interaction with the host, which are crucial in the synthesis, extraction and absorption of several nutrients and metabolites such as amino acid, lipids, bile acids, short chain fatty acids (SCFA) and vitamin (Khosravi & Mazmanian, 2013). Recent studies suggested that changes in the microbiota could be affected by the presence of *Blastocystis* sp. but result could be heterogeneous. Most of the time, risk factors for the acquisition of the infection similar in both immunocompetent and immunocompromised individuals. However, after exposure, immunocompromised patients are unable to clear the parasites, thus undergo a more serious or diffuse infection due to the poor protective barrier of these patients (Stark *et. al.*, 2010; Caner *et. al.*, 2020; Zanetti *et al.*, 2020). Compared to healthy individuals, a higher incidence of *Blastocystis* sp. has been observed in patients with significant symptoms that are linked to gastrointestinal signs such as abdominal pain, diarrhoea, nausea, flatulence and constipation. The parasite may also have a role in a number of chronic illnesses with unclear aetiology, such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), haemodialysis and others (Ajjampur & Tan, 2016; Shafiei *et al.*, 2020).

Although data on the prevalence of *Blastocystis* sp. in some regions are available, there is little information on the reviewing of the prevalence of *Blastocystis* sp. subtypes in clinical diseases especially in immunocompromised patients that had been reported worldwide. Considering the important of this topic, we hypothesised the potential relation between *Blastocystis* sp. subtypes prevalence and clinical diseases. In addition, the correlation of *Blastocystis* sp. infection could be demonstrated either pathogenically or symbiotically with gut microbiota.

II. METHODS

A. Exploration Resources

Literature review was conducted in September 2020 using two main databases, Scopus and PubMed. Another manual searching efforts by scientific electronic library (Scielo, Science direct and Springer) were also being used. It includes texts with abstracts or full databases, bibliographic

references as well as academic search engines (Google scholar) to enhance the relevant articles about the association of *Blastocystis* sp. In medical disorder especially in immunocompromised individuals. The search was restricted to articles published in English and only studies were carried out within 2010-2020 with open access had been chosen. This study includes investigations carried out in the past 10 years in order to present more recent and comprehensive study conducted on *Blastocystis* sp.

B. A Systematic Review Approach

1. Identification process

Keywords were recognised in the first stage of review. Initially, MEDLINE search was confined using MeSH index terms and associated keywords based on the thesaurus, dictionaries and previous researches. Therefore, search strings on Scopus and PubMed database were developed in September 2020 (Figure 1) after all relevant keywords accomplished to be determined including specific parasite and disease. Literature was completed using the terms; "*Blastocystis* sp.", "subtypes", "pathogenicity", "immunocompromised", "gut microbiota" and interchangeable words were used in this study such as "intestinal parasites", "virulent or infectious", "immunosuppression" and "disease" that were possible to have *Blastocystis* sp.'s infection. Then, a review of the abstracts of more than 300 publications was performed in the first stage.

2. Screening process

Several duplicate articles and irrelevant articles were removed in a screening phase. In this case, a total of 233 articles remained after the removal of duplicates and irrelevant articles in the first stage. The publications were screened according to inclusion and exclusion criteria, which were literature type focussing on the journal (full-length research articles) as it serves as the main source of empirical data. In the second stage, publication in the form of series, review, systematic review, meta-analysis, meta synthesis, books, chapter of books and conference or proceeding were omitted. Furthermore, the review only focused on publications from the last 10 years, from 2010 to September 2020; published articles in English with open and full text access. As a result, a total of 64 articles were remained based on inclusion and exclusion criteria.

3. Eligibility process

A total of 64 articles were organised for the third step in systematic literature review known as the eligibility. Importantly, the titles, abstracts, and primary contents of all the publications were thoroughly checked to ensure that they met the inclusion criteria and were appropriate for use in the current study in line with the implementation of the current research. Consequently, a total of 27 articles were discarded because the articles did not address the occurrence of *Blastocystis* sp. infection in the disease. Thus, a total of 37 remaining articles were ready to be analysed. An additional of 20 related articles by manual searching and using snowballing were included for analysis. Finally, 57 papers were discussed in this review.

C. Study Quality, Data Extraction and Analysis

The consistency of each study was evaluated by discussing the recorded specifics of the research among the authors. The pairs of reviewers have worked independently to determine the validity of the qualifying reports. In this study, PRISMA table was adopted by reporting a searching process consisting of identification, screening and eligibility process as be shown in Figure 1. The quality of demographic data, research location, sample size, study design, number of positives and diagnostic test were all evaluated using a data extraction table in Table 1. The observation onto *Blastocystis* sp. subtypes among targeted diseases also had been included in this study across the country and disease in Table 2. Additional studies was focusing on the association of *Blastocystis* sp. subtypes in the gut microbiota in Table 3.

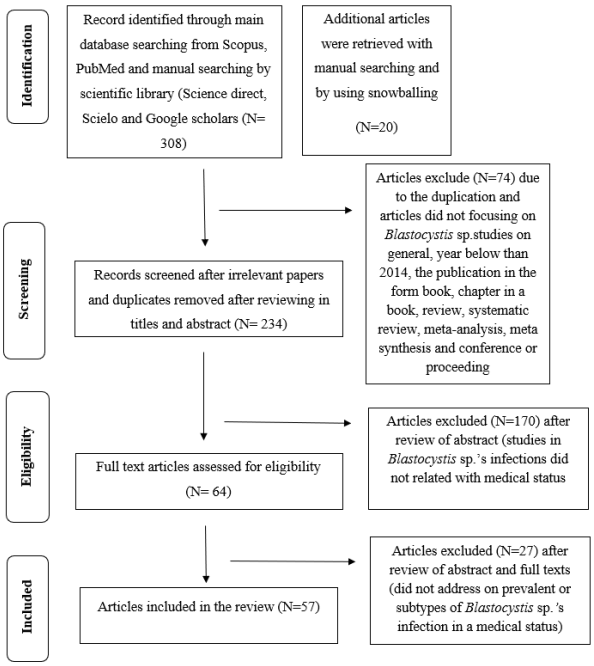

Figure 1. Flow diagram of search strategy and selection process

III. RESULTS AND DISCUSSION

A. General Study Characteristic

A systematic review was performed on the available literatures to identify the association between *Blastocystis* sp. subtypes in the clinical disease in conjunction with colonisation towards gut microbial community. A total of 57 articles were examined thoroughly from 2010 to October 2020 regarding the prevalence of *Blastocystis* sp. subtypes in immunocompromised patients and the relationship between of *Blastocystis* sp. subtypes and gut communities. The common diseases or immunological status that were associated with *Blastocystis* sp. such as, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) or chronic diarrhoea, HIV/AIDS and cancer were widely reported in several countries. In this review, 13 articles discussed on *Blastocystis* sp. infection in IBS patients, 11 articles on HIV patients, five articles on cancer patients and six articles on diabetic patients. Other articles pertaining to disease or immunological status such as dengue fever, post cardiac patients, transplant patients, haemodialysis patients, post traumatic and leukaemia patients were also highlighted in this review. Infection with *Blastocystis* sp. discovered in recent studies involving symptomatic patients and asymptomatic individuals are been summarised in Table 1. Regarding the distribution of studies based on the medical status, most studies demonstrated that immunocompromised patients have the higher prevalence of infection by *Blastocycstis* sp. as compared to healthy individuals. Among 11 review studies from IBS, six studies showed that the highest detection of *Blastocystis* sp. in male IBS patients while only two review studies highlighted in control group had higher prevalence of *Blastocystis* sp. infection. Two review papers showed that *Blastocystis* sp. infection was associated with gender as the male IBS has higher rate of infection compared to female (Krogsgaard *et. al.*, 2015; Beiromvand *et al.*, 2017). From the 10 review articles, only one indicated lower prevalence of *Blastocystis* sp. infection in HIV positive than HIV negative patients. Most studies demonstrated that higher detection of *Blastocystis* sp. infection in positive HIV patients was connected with CD4 T-cell count (Ghimire *et. al.*, 2016; Zhang *et al.*, 2019). Meanwhile, in the five reviewed articles for cancer diseases, one case control study reported that *Blastocystis* sp. had been detected with higher occurrence in cancer patients compared to healthy individuals which was associated with the cycle of chemotherapy treatment received. Moreover, in six reviewed articles of diabetes mellitus, the most common intestinal parasite was *Blastocystis* sp. with a significant difference between diabetes mellitus (DM) group and control group. A total of 16 papers were reviewed on other diseases included IBD, pulmonary tuberculosis, acute lymphocyte, leukaemia, haemodialysis and dengue. Among that, only two case control studies were reported to have higher prevalence of *Blastocystis* sp. infection in immunosuppressed patients than control group. A phlylogenetic analysis of nucleotide data from small subunit ribosomal DNA (SSU rDNA) was used in most studies to identify *Blastocystis* sp. subtype in samples from different medical condition and healthy individuals (Table 2 and 3). Among these studies, *Blastocystis* sp. ST3 showed the highest prevalence and was common among immunocompromised patients followed by ST1 and ST2. One study showed ST1 was more prevalent in HIV patients. In addition, 12 studies revealed that there was a significant difference in microbial taxa between *Blastocystis* sp. colonisation in gut microbiota and

Blastocystis sp. free among the faecal samples that were collected from humans and mice as summarised in Table 4.

B. Prevalence of Blastocystis sp. Infection in Clinical Diseases

Blastocystis sp. is presumably the most frequent protozoan observed in human faecal samples of both symptomatic and asymptomatic individuals worldwide. The prevalence of *Blastocystis* sp. in immunocompromised individuals varies from various region of the world with unknown specific mechanism. Irritable bowel syndrome (IBS) is one of the common diseases that had been revealed to be linked with *Blastocystis* sp. infection. IBS is a digestive disorder with symptoms like constipation, abdominal pain and diarrhoea. In nine IBS reviewed studies, six articles reported that the prevalence of *Blastocystis* sp. infection in IBS patients was higher compared to healthy control (Surangsrirat *et. al*., 2010; Ramirez-Miranda *et. al*., 2010; Nourrisson *et. al*., 2014; Vargas-Sanchez *et. al*., 2015; Das *et. al*., 2016; Jadallah *et. al*., 2017; Kesuma *et. al*., 2019; Shafiei *et al*., 2020). It was suggested that there were several risk factors of developing IBS including agent factors such as the nature of the pathogen (bacterial, parasites, virus), host-related factors (genetics, age, sex), dietary factors physiological state (depression, anxiety, etc) (Cifre *et al*., 2018). *Blastocystis* sp. infection was significantly correlated with gender where *Blastocystis* sp. infection was more prevalent in IBS male patients (Kesuma *et. al*., 2019; Peña *et al*., 2020). However, several other studies revealed that *Blastocystis* sp. had higher prevalence in healthy individuals than IBS patients. It was proposed that the protozoan could play a protective role in IBS (Krogsgaard *et. al*., 2015; Beiromvand *et al*., 2017).

Blastocystis sp. has been well documented as one of the common enteric protozoa in HIV-infected patients due to weaken immunity (Adarvishi *et al*., 2016). According to a study in Rome among 156 HIV positive patients, microscopic examination detected the presence of *Blastocystis* sp. in 34 patients (21.8%) whereas molecular analysis revealed this protist in 39 individuals (25%). It has been observed that *Blastocystis* sp. was reported to have higher prevalence in men who have sex with men (MSM) compared to the heterosexuals due to the faecal-oral contact as the main route of transmission. This has been associated with *Blastocystis* sp. infection by sexual practice and life style more than the immunological status (Gabrielli *et al*., 2020). Besides, three reviewed papers in case control and cross-sectional studies demonstrated that the prevalence of *Blastocystis* sp. infection in HIV patients were higher than healthy individuals with prevalence rate recorded at 15% in Nepal ,77.8% in Indonesia, 19% in Iran for HIV patients, 22% and zero detection respectively in healthy control (Ghimire *et. al*., 2016; Piranshahi *et. al*., 2018; Darlan *et al*., 2020). HIV positive disease often been observed by CD4+ T cell count as a predictive tool of the infection and linked with the incidence as the severity of infection (Nissapatorn and Sawangjaroen, 2011). Higher prevalence of *Blastocystis* sp. had been associated with lower CD4 T+ in the HIV- positive (Ghimire *et. al*., 2016; Piranshahi *et. al*., 2018; Zhang *et al*., 2019). In addition, *Blastocystis* sp. was found to be one of the major intestinal parasites detected in the study from Iran, which was about 10.9% compared to *Giardia lambia*, *Entamoeba coli* and others (Adarvishi *et al*., 2016). Contradictorily, the study by Cristanziano *et al*. (2019) in Ghana discovered that the prevalence of *Blastocystis* sp. infection in HIV positive patients was lower compared to non-HIV infected individuals. It has been counted by CD4+ T cell where the positive *Blastocystis* sp. infection in HIV patients had higher CD4+ T cell count than free *Blastocystis* sp. co-infection in HIV patients. Thus, this study inversely suggested an association of *Blastocystis* sp. with a better of immune status that had be explained in the comparison of CD4+ T cell count among HIV positive patients. In HIV-negative participants, *Blastocystis* sp. also has been observed to have an association with healthy body weight (Cristanziano *et al*., 2019).

Among five reviewed studies in cancer disease, *Blastocystis* sp. demonstrated a higher prevalence rate compared to other parasites and there was no detection of *Blastocystis* sp. in one case control study in healthy individuals (Yersal *et. al*., 2016; S. Rasti *et. al*., 2017; W. Zhang *et. al*., 2017; Esteghamati *et. al*., 2019; Asghari *et al*., 2020). Data from several studies identified that *Blastocystis* sp. infection rate was directly related to those who were in chemotherapy group. As more chemotherapy cycles were received by the patients, they are more likely to be infected

by *Blastocystis* sp. (Zhang *et. al.*, 2017; Asghari *et al.*, 2020). In addition, research on diabetes mellitus were also reviewed in six articles which showed that there was a statistical difference between the prevalence of intestinal parasites in the diabetic group and the non-diabetic group with *p*-value <0.05 with *Blastocystis* sp. being one of the highest detected parasites (Mohtashamipour *et. al.*, 2015; Fattahi Bafghi *et. al.*, 2015; Tangi *et. al.*, 2016; Ali *et al.*, 2018). Women with diabetes showed a higher prevalence of parasitic infection including *Blastocystis* sp., *Endolimax* nana and *Giardia lambia* as compared to men with diabetes (Mohtashamipour *et. al.*, 2015; Ali *et al.*, 2018). Meanwhile, few other studies in patients with cancer reported inverse findings in which *Blastocystis* sp. infection was significantly higher in male patients compared to female with cancer (Yersal *et. al.*, 2016; Rasti *et al.*, 2017).

Besides, *Blastocystis* sp. infection was also observed in other immunocompromised patients such as ulcerative colitis, pulmonary tuberculosis (PTB), transplant recipient, lymphotic leukaemia, heamodylisis, irritable bowel disease (IBD), post cardiotomy patient, post traumatic splectnectomised and dengue (Table 1). Several studies demonstrated higher infection of *Blastocystis* sp. were detected in patients under heamodialysis and post traumatic splenectomised as compared to healthy individuals (Karasartova *et. al.*, 2016; Rasti *et al.*, 2017) while five studies reported an inverse finding. Interestingly, the prevalence of *Blastocystis* sp. infection in dengue patients was higher and had been correlated significantly with high fever, low platelet count and longer admission duration than the non-*Blastocystis* sp. infected dengue patients (Thergarajan *et al.*, 2019). *Blastocystis* sp. infection was substantially linked with gender with higher detection in male patients (Rasti *et. al.*, 2017; Thergarajan *et al.*, 2019). Most studies indicated that *Blastocystis* sp. often causes opportunistic infection in immunocompromised patients, especially diarrhoea that usually accompanied by several symptoms including bloating, abdominal pain, constipation, flatulence and in the some of the cases, fever (Beyhan *et. al.*, 2015; Safadi *et al.*, 2016). Specifically in dengue fever, a study found that gastrointestinal indications among dengue patients could lead to *Blastocystis* sp. infection, where there has been a rise in the time of hospitalisation for *Blastocystis* sp.-infected dengue patient (Thergarajan *et al.*, 2019). Nevertheless, the dispersion of the result would be different, which could impact many variables, including the health status of the area, the target groups surveyed, the methods of sampling and techniques applied for identification. Overall, the reviewed studies demonstrated that prevalence data are highly influenced by the diagnostic approach used; the most common methods used in routine diagnosis are microscopic methods such as trichome staining and native lugol, while culture and molecular methods are preferred for research purposes. There was a clear difference in the detection of *Blastocystis* sp. positive cases using PCR and microscopic methods, as well as culture and microscopic approach in the reviewed studies.

C. *Blastocystis sp. Subtypes and Clinical Diseases*

The pathogenesis of *Blastocystis* sp. remains ambigous as the infection can be observed in both immunocompromised patients and healthy individuals based on persistent information gaps in epidemiology factor, influencing host colonisation and interaction with host, both direct and via the gut ecosystem (Chabé *et al.*, 2017). Subtyping has been related to infection of *Blastocystis* sp. in immunocompromised patients and healthy individuals for several years. The prevalence of subtypes appears to differ between countries and within populations of the similar state. ST3 was more abundant and common in the immunocompromised patients; IBS, transplant candidates, HIV patients, cancer, dengue fever and IBD patients followed by ST1 and ST2. Meanwhile, ST4 is common in Europe and was also detected in the population of IBS, IBD and HIV (Table 2). Notabaly, ST3 has high intra-subtype diversity and is likely to co-evolve with human hosts over a longer time span compared to ST1, ST2 and even ST4, which could have colonised humans at various times across an evolutionary time scale (Scanlan and Stensvold, 2013). In two IBS reviewed studies, *Blastocystis* sp. ST1 is the most prevalent where it was associated with IBS-diarrhoea with a risk factor of 2.9 times, indicating that *Blastocystis* sp. ST1 is likely to be correlated with the clinical presentation of diarrhoea and probably is a pathogenic strain (Vargas-Sanchez *et. al.*, 2015; Kesuma *et al.*, 2019). Contradictory, Das *et al.* (2016) reported that ST3 was higly detected

instead of ST1 in IBS patients compared to controls. From these studies, it is noted that there is no particular subtype that is linked to any particular clinical type of IBS. Interestingly, Vargas-Sanchez *et al.* (2015) indicated that the generation times of *Blastocystis* sp. could be easily influenced by intestinal environmental changes due to IBS possibly because virulent strains with slow growth may be selected, reducing their genetic variability. Furthermore, in one reviewed study of other diseases by Bednarska *et al.* (2018) observed that *Blastocystis* sp. ST3 and ST2 were detected in individuals who suffered from intestinal disorder and colitis ulcerosa respectively. ST3 is associated with weight loss while ST2 with diarrhoea and abdominal pain. ST3 was also reported in a patient with splenic cysts who had localised pain in left hypochondrium (Santos *et al.*, 2014). In a dengue study in Malaysia by Thergarajan *et al.* (2019), low prevalence of *Blastocystis* ST4 and ST6 were reported. Meanwhile, ST7 was observed in Mexico, Brazil, Rome and Iran with a lower distribution (Vargas-Sanchez *et. al.*, 2015; Ali Asghari *et. al.*, 2020; Gabrielli *et. al.*, 2020; Silva *et al.*, 2020). As *Blastocystis* ST3 being the most documented subtype in symptomatic and asymptomatic patients, further research needs to be undertaken in order to obtain better understanding on the genotypic, phenotypic and pathophysiological aspects of *Blastocystis* sp. subtypes.

D. Association Blastocystis sp. Subtypes in The Gut Microbiota

Blastocystis sp. has been characterised as an intestinal parasite that is either a commensal or pathogen depending on the subtypes. It is proposed that different subtypes of *Blastocystis* sp. could colonise in different niches, which would indicate diverse potential interactions with specific human gut microbial ecosystems. From the reviewed papers, *Blastocystis* sp. can be observed as an indicator to improve gut microbiota balance (eubiosis) and can alter the changes in the intestinal flora itself quantitatively and qualitatively where it leads to microbial imbalance (dysbiosis).

1. The adverse associations of Blastocystis sp. on gut the microbiota

Alteration of gut microbiota dysbiosis could be linked to the presence of *Blastocystis* sp., which would lead to a number of diseases including gastrointestinal disorders with mysterious mechanism. Dysbiosis or imbalanced microbiome is characterised by pathobiont overgrowth, commensal loss and reduced diversity where it may lead to association with the aetiology of several diseases (Levy *et al.*, 2017). The composition of an individual's gut microbiota can fluctuate under some circumstances such as acute diarrhoea, antibiotic treatments, psychological stress, modern diet and hygiene which was typified by a decrease in bacterial diversity, a reduction in *Clostridia* abundance and an increase of facultative anaerobic Gammaproteobacteria like Enterobacteriaceae (Hawrelak & Myers, 2004; Bernstein & Shanahan, 2008; Winter & Bäumler, 2014; Defaye *et al.*, 2020). According to a reviewed study from Nagel *et al.* (2016), there was a change in the faecal microbiota in the main phyla with the rise of Firmicutes and statistically significant reduction in Bacteroidetes relative abundance were in diarrhoea-predominant IBS patients than healthy individuals. The Firmicutes/Bacteroidetes (F/B) ratio is commonly considered to have an essential influence in sustaining normal intestinal homeostasis and the change of the ratio can lead to dysbiosis. For instance, it is usually observed in inflammatory bowel disease (IBD) and obesity (Stojanov *et al.*, 2020). However, there is no significant difference of *Blastocystis* sp. carriage seen in the phyla or genus profile of the gut microbiota among IBS patients. Moreover, an article reported *Blastocystis* sp. colonisation was correlated with significant changes in bacterial alpha and beta diversity, which significantly showed difference in the abundances of predominant bacterial taxa, including *Prevotella stercorea*, *Prevotella copri*, *Alistipes putredinis, Ruminoccoccus bromii*, *Bacteroides* sp., *Oscillospira* sp. and *Bifidobacterium longum*. It has been explained by a decline in short-chain fatty acid (SCFA) levels in faecal samples from asymptomatic patients who were infected by *Blastocystis* sp. which leads to a reduction in the beneficial pathways including a carbohydrate fermentative metabolism by *Prevotella copri*. In *Blastocystis*-infected individuals, the three main SCFA; acetate, butyrate and propionate were reduced while the caprote was increased. Furthermore, *Blastocystis* sp. ST3 was shown to be the most significantly different in bacterial community abundance between *Blastocystis* sp. positive and negative individuals (Nieves-

Ramirez *et al.*, 2018). Several *in vivo* rodent experiments revealed the pathogenicity which involved a rare subtype, ST7 and the common, ST4 displays minor inflammation and pathology to host's tissue. A study using Wistar rat reported that there was a significantly greater change in bacterial richness in chronically infected rats by 10^5 ST4 cysts, which was observed in increasing relative abundance of *Tenericutes* and *Proteobacteria* in infected animals by *Blastocystis* sp. In addition, the decrease of Firmicutes, Bacteroidetes, *Clostridium* with increasing *Oscillospira*were significantly correlated with colonic hypersensitivity. A decrease in the overall content of SFCAs in the feaces composition of infected rats was observed (Defaye *et al.*, 2020). *Clostridium*, a member of the Clostridiaceae family was also identified as a SCFA-producing bacteria (Hugenholtz *et al.*, 2018). SCFAs are important metabolites in the maintenance of intestinal homeostasis that enhance epithelial barrier function and immune tolerance and have been produced dominantly by Bacteoidetes (gram negative) and Firmicutes (gram-positive) in the human gut (Rooks & Garrett, 2016; Venegas *et al.*, 2019).

Similarly, in an *in vivo* and *in vitro* study conducted by Yason *et al.* (2019) demonstrated that *Blastocystis* sp. ST7 infection in mice reduced the population of beneficial bacteria (*Bifidobacterium* sp. and *Lactobacillus* sp.) with an increase in the population of enterobacteria (*E. faecalis, Escherichia coli, B. subtilis* and *B. fragilis*) in the faecal samples. In this case, it had negatively affected beneficial bacterial population, which contributed in the production of other SCFAs that produce acetate and lactate during carbohydrate fermentation (Rivière *et al.*, 2016). *Lactobacillus* sp. reduction was greater in ST7-B infected mice, whereas *E.coli* growth was greater in ST7-H infected mice. It was indicated that ST7-B tended to be a better carrier of dybiotic harm than ST7-H, resulting to be more severe binding to the host cells (Yason *et al.*, 2019). *Lactobacillus* sp. and *Bifidobacterium* also belong to the probiotic bacteria in the gut that have anti-inflammatory role and could significantly increase IgA level. Although some *Lactobacillus* sp. strains have been associated to sepsis, especially bacteria belonging to *Lactobacillus* genus, they can inhibit pathogenic microorganism proliferation and control immune responses that promote intestinal and systemic health (Levy *et. al.*, 2017; Yelin *et al.*, 2019). However, due to the alteration of both intestinal bacteria in modulating T-cell response by dendritic cells through the increased production of IL4 and the decreased excretion in IL-22 and IFN- γ, an aspect of intestinal epithelium defence will be eliminated to help in the pathogenesis of *Blastocystis* sp. (Mann *et. al.*, 2014; Yason *et al.*, 2019).

In addition, the association between the presence of *Blastocystis* and *Clostridium difficile* infection in diarrhoeic patients has been reported by Vega *et al.* (2020), indicating a reduction in beneficial becteria while increasing the abundance of bacteria belonging to the Enterobacteriacea family or other groups of bacteria pathogens. It has been suggested that *Blastocystis* sp. may be able to adapt to dysbiosis and oxidative stress (Vega *et al.*, 2020). This was in line with Nourrisson *et al.* (2014) who suggested that *Blastocystis* sp. might be correlated to the pathophysiology of IBS-C and intestinal flora imbalance since the result showed a reduction of beneficial bacteria in *Blastocystis*-positive patients with IBS. Overall, changes in the feasibility of these bacterial species could lead to potential disturbances in the intestinal community which could have a major impact on a number of inflammatory cases in clinical diseases, especially in IBS and IBD.

2. The beneficial associations of Blastocystis sp. on gut the microbiota

Despite of *Blastocystis* sp. being suggested as one of the possible parasites that causes dysbiosis, *Blastocystis* sp. colonisation also has been allied to eubiotic state, categorised by a dominance of potentially beneficial species primarily belonging to the phyla Firmicutes and Bacteoidetes instead of those of the phylum Protebacteria. Higher bacterial diversity of faecal microbiota comes from *Blastocystis* sp. colonised individuals rather than *Blastocystis*-free individuals. There were higher abundance of Clostridia class, Ruminococcacea and Provotellaceafamilies in *Blastocystis* infected individuals while Enterobacteriacaea was enriched in *Blastocystis*-free patients (Audebert *et al.*, 2016). Moreover, Gabrielli *et al.* (2020) validated the results of greater bacterial diversity in *Blastocystis* sp. ST3 carriers compared to free-*Blastocystis* sp. individuals. It was shown that *Blastocystis* sp. was found

mostly in immunocompromised individuals, especially HIV patients, due to the increase bacterial diversity such as Prevotellaceae, Methanobacteriaceae, Clostridiaceae, Lachnospiraceae, Erysipelotrichaceae and Pasteurellaceae and low level of Bacteroidaceae and Veillonecel in the family level that strongly promotes colonisation by the protist. An early approach by metagenomic study also found that individuals that were dominated by beneficial bacteria such as *Ruminococcus* and *Prevotella* enterotypes have been linked to the presence of *Blastocystis* sp. in the gut (Andersen *et al*., 2015). Besides, *Blastocystis* sp. has also a significant association with high abundance of *Prevotella* and a lower abundance of *Clostridial* cluster XIVa and *Bacteroides* (Andersen *et al*., 2016).

Moreover, *Blastocystis* sp. subtypes were strongly linked to community of microbiota with higher explanatory power in a non-clinical cohort and has been connected to both *Blastocystis* sp. prevalence and subtype variation (Tito *et al*., 2019). Beyond this association, there was a substantial difference between ST3 and ST4 with ST4 being more abundant in the highest diversitiy of Ruminococcaceae entrotyped samples among *Blastocystis* sp. carriers. ST2 was found to be more common than ST3 in Ruminococcaceae samples compared to *Prevotella* enterotype samples, whilst ST4 was shown to be positively associated with Methanobrevibacter relative abundances. Although the high richness and enterotyped distribution observed in *Blastocystis* sp. carriers showed that it was a common member of stable, resilient and health associated microbiota, a subtype level analysis revealed the crucial differences within the genus. Remarkably, *Akkermansia* relative abundances was observed to be correlated negatively with abundance of *Blastocystis* sp. ST3 and positively with ST4. As observed, when dysbiosis occurs, the level of *Akkermansia munciniphilla* decreases and there was an increased risk of ST3 infection, which is closely linked to IBS that explains the occurrence of gastrointestinal indications. In contrast, the growth of these bacteria is correlated with the presence of ST4. This study found that ST4 could be linked to markers of healthy gut microbiota such as *Akkermansia,* which is typically regarded as a health-promoting GI isolate (Tito *et al*., 2019).

In a paper which focuses on healthy Malian children stated that *Blastocystis* sp. colonisation has a significant correlation with a higher bacterial diversity in the gut when there is a rise of Firmicutes, Elusimicrobia, Lentisphaerae, and Euryarchaeota in phyla level while it is not correlated with the existence of potentially pathogenic bacteria in the human gut. More precisely, this study discovered that *Roseburia* sp. (family Lachnospiraceae) and *Faecalibacterium prausnitzii* (family Ruminococcaceae) were relatively more abundant in children colonised by *Blastocystis* sp. where they had essential role in gut physiology, digesting dietary fibre into short chain fatty acids for energy sources and possessing anti-inflammatory properties (Kodio *et. al*., 2019; Lordan *et al*., 2020). In general, the reviewed data revealed that *Blastocystis* sp. is also a part of a stable intestinal microbiota as evidenced by higher bacterial diversity and a reduced incidence of inflammatory disorders (Loh & Blaut, 2012).

IV. CONCLUSION

Blastocystis sp. has been widely described particularly in immunocompromised individuals with positive association in many diseases. Generally, these review studies showed that *Blastocystis* sp. infection was not solely associated with the risk factors of demographic characteristics and molecular detection by PCR would be the best method to identify *Blastocyctis* with the higher specificiation of data. Based on summary, *Blastocystis* sp. may be a part of a stable intestinal microbiota and particular isolates or unusual subtype may interfere with homeostasis contributing to dysbiosis in the host. Yet, numerous studies are still uncertain about the differentation of *Blastocystis* sp. subtypes whether it influence the gut microbial composition or vice versa as the same subtypes may act as commensal or a pathogen depending on unclear specific conditions. Therefore, studies on the evaluation of *Blastocystis* sp. on subtypes level should be conducted in humans or other naturally occurring hosts. In-depth insight, these data could facilitate future studies for a better understanding on these issues revolve around pathogenesis, clinical symptoms and prevalence of *Blastocystis* sp. subtypes in immunosuppressed patients compared to healthy individuals in line with the richness and diversity of gut

microbiota in the presence of this protist. With this in mind, the clear picture of this parasitic infection can act as a baseline data and may help to increase comprehension of its clinical diagnosis.

V. CONFLICT OF INTEREST

No conflict of interest among all authors.

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Table 1. Studies on *Blastocystis* sp. in immunocompromised patients & healthy individuals (2010 – 2020)

Immunological/ medical status	Study area	Study design	Total number of tests	Prevalence (%)		Diagnostic method	Reference
				Patients	Healthy individuals		
Irritable bowel syndrome (IBS)	Chile	NA	37	10.81%	NA	Microscopy & PCR	(Peña *et al.*, 2020)
	Iran	Case control	200	15%	6%	Microscopy	(Shafiei *et al.*, 2020)
	Indonesia	Cross sectional	454	36.5%	28.6%	Microscopy, culture & PCR	(Kesuma *et al.*, 2019)
	Iran	Case control	282	1.3% 28.6% 27.3%	12.3% 71.4% 72.7%	Microscopy culture PCR	(Beiromvand *et al.*, 2017)
	Jordan	Case control	209	25.7%	9%	PCR	(Jadallah *et al.*, 2017)
	France	Case control	112	23.2%	16.1%	PCR	(Nourrisson *et al.*, 2014)
	Mexico	Case control	100	49%	48%	Culture & PCR	(Vargas-Sanchez *et al.*, 2015)
	Denmark	Case control	483	15%	22%	Microscopy & culture	(Krogsgaard *et al.*, 2015)
	India	Case control	250	33.3%	15%	PCR	(Das *et al.*, 2016)
	Thailand	Case control	126	16.7%	10.0%	Culture	(Surangsrirat *et al.*, 2010)
	Pakistan	Case control	315	70%	29%	PCR	(Yakoob *et al.*, 2010)
	Mexico	Case control	324	15.7%	12.0%	Microscopy	(Ramirez-Miranda *et al.*, 2010)
IBS, IBD & acute diarrhoea	Rome	Cross sectional	2524	22.3%	6.8%	Microscopy & PCR	(Gabrielli *et al.*, 2020)
	Turkey	Case control	2334	5.74%	3.12%	Microscopy	(Cekin *et al.*, 2012)
HIV/AIDS	Ghana	Case control	192	6.6%	20%	PCR	(Cristanziano *et al.*, 2019)
	China	Cross sectional	311	3.86%	NA	PCR	(Zhang *et al.*, 2019)
	Rome	Cross sectional	156	25%	NA	PCR	(Fontanelli Sulekova *et al.*,

				21.8%		Microscopy	2019)
	Indonesia	Cross sectional	52	77.8%	22.2%	Microscopy	(Darlan *et al.*, 2020)
	Iran	Cross sectional	268	12.3% 19%	NA	Microscopy PCR	(Piranshahi *et al.*, 2018)
	Iran	Case control	140	15.0%	0%	Microscopy	(Rasti *et al.*, 2017)
	Nepal	Cross sectional	112	2.2%	NA	Microscopy	(Ghimire *et al.*, 2016)
	Burkina Faso	Cross sectional	291	1.0%	NA	Microscopy	(Sangaré *et al.*, 2015)
	Iran	Cross sectional	200	10.9%	NA	Microscopy	(Adarvishi *et al.*, 2016)
	Laos	Cross sectional	137	26.3%	NA	Microscopy	(Paboriboune *et al.*, 2014)
	Indonesia	Cross sectional	42	96%	NA	Culture	(Idris *et al.*, 2010)
Cancer	Iran	Cross sectional	190	22.3%	NA	PCR	(Esteghamati *et al.*, 2019)
	Iran	Cross sectional	200	6.5% 10.5% 12%	NA	Microscopy Culture PCR	(Asghari *et al.*, 2020)
	Turkey	Cross sectional	232	6.5% 10.8%	NA	Microscopy Culture & PCR	(Yersal *et al.*, 2016)
	China	Cross sectional	381	7.1%	NA	PCR	(Zhang *et al.*, 2017)
	Iran	Case control	122	3.3%	0%	Microscopy	(Rasti *et al.*, 2017)
Diabetes mellitus	Iraq	Cross sectional	419	35.48%	NA	Microscopy	(Ali *et al.*, 2018)
	Iran	Case control	236	9.3%	6.8%	Microscopy	(Mohtashamipour *et al.*, 2015)
	Cameroon	Cross sectional	235	2.7%	1.2 %	Microscopy	(Tangi *et al.*, 2016)
	Iran	Cross sectional	500	2.4%	2.4%	Microscopy	(Fattahi Bafghi *et al.*, 2015)
	Iran	Case control	501	9.1%	10.3%	Microscopy	(Poorkhosravani *et al.*, 2019)
	Egypt	Cross sectional	185	20%	NA	Microscopy	(Esmail *et al.*, 2019)
Acute Diarrhoea	Qatar	Cross sectional	580	4.7%	NA	PCR	(Boughattas *et al.*, 2017)
Ulcerative colitis (UC)	Netherland	Case control	168	13.3%	32.5%	Microscopy	(Rossen *et al.*, 2015)
	Denmark	Retrospective	316	14.9%	20.3%	PCR	(Andersen *et al.*, 2015)

	Belgium	Cohort study	1168	4%	30%	PCR	(Tito *et al.*, 2019)
Post traumatic splenectomised	Turkey	Case control	60	30% 40%	13% 13%	Microscopy PCR	(Karasartova *et al.*, 2016)
Lymphocytic Leukaemia	Egypt	Case control	101	54.5%	67.4%	Microscopy	(Eassa *et al.*, 2016)
Immunocompetent/ transplant recipient/medical suppression	Poland	Case control	283	1.2%	NA	PCR	(Bednarska *et al.*, 2018)
	Iran	Case control	50	0%	0%	Microscopy	(Rasti *et al.*, 2017)
	Iran	Cross sectional	80 25	16.25% 0%	NA	Microscopy & PCR	(Esteghamati *et al.*, 2019)
	Turkey	Cross sectional	62	9.6%	NA	Microscopy	(Caner *et al.*, 2020)
Renal, hepatic & bone marrow transplantation	Brazil	Retrospective	150	16%	NA	PCR	(Silva *et al.*, 2020)
Post cardiotomy patient	Brazil	Case report	1	100%	NA	Microscopy & PCR	(Santos *et al.* 2014)
	Taiwan	Case report	1	100%	NA	Culture	(Chen *et al.*, 2014)
	Iran	Case control	155	4.4%	0%	Microscopy	(Rasti *et al.*, 2017)
Haemodialysis	Brazil	Cross sectional	196	24.5%	41.9%	Microscopy	(Gil *et al.*, 2013)
Pulmonary Tuberculosis (PTB)	China	Cross sectional	775	6.2%	7.6%	Microscopy	(Li *et al.*, 2014)
Dengue	Malaysia	Cross sectional	89	23.6%	NA	Culture & PCR	(Thergarajan *et al.*, 2019)

NA; Not available, PCR; Polymerase chain reaction

Table 2. Subtypes distribution of *Blastocystis* sp. identified in immunocompromised individuals with diseases (2010 – 2020)

Immunological/ medical status	**Study area**	**Subtypes distribution of *Blastocystis* sp. (%)**							**References**
		ST 1	**ST2**	**ST3**	**ST4**	**ST 6**	**ST 7**	**Mixed**	
Inflammatory bowel syndrome (IBS)	Chile	NA	NA	-	NA	-	-	-	(Peña *et al.*, 2020)
	Indonesia	10.9%	1.5%	10.2%	-	-	-	-	(Kesuma *et al.*, 2019)
	Iran	3.7%	11.1%	11.1%	-	-	-	-	(Beiromvand *et al.*, 2017)
	Mexico	41%	24%	33%	-	-	2%	-	(Vargas-Sanchez *et al.*, 2015)
	France	1.78%	3.57%	5.35%	10.71%	-	-	1.78%	(Nourrisson *et al.*, 2014)
	Pakistan	86%	60%	47%	75%	50%	50%	-	(Yakoob *et al.*, 2010)
	Egypt	29.4%	-	43.14%	15.69%	-	-	11.77%	(Fouad *et al.*, 2011)
	India	6%	-	94%	-	-	-	-	(Das *et al.*, 2016)
Transplant	Brazil	37.5%	12.5%	45.8%	-	-	4.2%	-	(Silva *et al.*, 2020)
HIV	Ghana	19%	9.5%	9.5%	-	-	-	-	(Cristanziano *et al.*, 2019)
	Rome	30.8%	7.7%	51.3%	10.2%	-	-	-	(Fontanelli Sulekova *et al.*, 2019)
	Iran	21.6%	11.8%	56.8%	3.9%	-	-	5.9%	(Piranshahi *et al.*, 2018)
Post-traumatic splenectomised	Turkey	10%	-	10%	-	-	-	-	(Karasartova *et al.*, 2016)
Cancer	Turkey	23%	18%	59%	-	-	-	-	(Yersal *et al.*, 2016)
	China	66.7%	-	40.0%	-	-	-	-	(Zhang *et al.*, 2017)
	Iran	20.9%	33.3%	37.5%	-	-	8.3%	-	(Asghari *et al.*, 2020)
Immunocompromised patients (IBS, IBD, acute diarrhoea)	Rome	29%	16%	40%	12%	-	3%	-	(Gabrielli *et al.*, 2020)
Ulcerative colitis (UC)	Denmark	2.99%	1.49%	2.99%	5.97%	1.49%	14.93%	-	(Andersen *et al.*, 2015)
Dengue	Malaysia	33.3%	1.5%	62%	14.3%	4.76%	-	-	(Thergarajan *et al.*, 2019)
Immunocompetent with pain in the left hypochondrium	Brazil	-	-	NA	-	-	-	-	(Santos *et al.*, 2014)

NA; Not available (The prevalence is not mentioned)

Table 3. Subtypes distribution of *Blastocystis* sp. identified in healthy individuals (2010 – 2020)

Study area	**Subtypes distribution of *Blastocystis* sp. (%)**							**References**
	ST 1	**ST2**	**ST3**	**ST4**	**ST 6**	**ST 7**	**Mixed**	
Chile	NA	NA	-	NA	-	-	-	(Peña *et al.*, 2020)
Indonesia	7.1%%	-	10%	-	-	-	-	(Kesuma *et al.*, 2019)
Iran	7%	13%	19%	-	-	-	-	(Beiromvand *et al.*, 2017)
Mexico	21%	23%	54%	-	-	-	-	(Vargas-Sanchez *et al.*, 2015)
France	-	-	-	8%	-	-	-	(Nourrisson *et al.*, 2014)
Pakistan	86%	60%	47%	75%	-	-	50%	(Yakoob *et al.*, 2010)
Egypt	-	26.53%	34.7%%	30.61%	-	-	-	(Fouad *et al.*, 2011)
India	3%	-	12%	-	-	-	-	(Das *et al.*, 2016)
Ghana	47.6%	9.5%	4.76%	-	-	-	-	(Cristanziano *et al.*, 2019)
Denmark	2.54%	4.24%	4.24%	7.20%	2.12%	-	-	(Andersen *et al.*, 2015)

Table 4. Studies on association of *Blastocystis* sp. colonisation with bacterial community between *Blastocystis* positive and *Blastocystis* negative infection in humans and mice

Subtypes	**Method**	**Diversity assessment**		**Gut microbiota composition shift**	**Status**	**References**
		α-diversity	***β*-diversity**			
***Blastocystis* (ST1-4,7)**	Amplicon-based NGS	Species richness, Chao-1 richness index	Principal Coordinate Analysis (PCoA) of weighted UniFrac distances	• INC bacterial richness in immunocompetent compared to immunocompromised patients. • INC in Firmicutes, Bacteroidetes and Proteobacteria (Phylum). • INC Bacteroida (Class) in *Blastocystis* sp. free subjects. • INC in Prevotellaceae, Methanobacteriaceae, Clostridiaceae Lachnospiraceae, Erysipelotrichaceae and Pasteurellaceae (Family). • DEC in *Bacteroidaceae* and *Veillonecel* (Family). • INC in *Prevotella*, *Methanobrevibacter*, *Ruminococcus*(Genus) in	Eubiosis	(Gabrielli *et al.*, 2020)

				Blastocystis carrier; INC in *Bacteroides*(Genus) in free *Blastocystis* sp.		
***Blastocystis* sp.**	Amplicon-based NGS	Shannon, Simpson Evenness, Species richness, Chao-1	Bray- Curtis dissimilarities, Principal Coordinate Analysis (PCoA) of Unweighted UniFrac distances	• INC bacterial diversity in *Blastocystis* carrier of healthy children • INC in Firmicutes, Elusimicrobia, Lentisphaerae, Euryarchaeota, *and* IHU_PP_Bacteria (Phylum) in *Blastocystis* carrier; INC Actinobacteria, Proteobacteria, unassigned bacteria, and Deinococcus–Thermus (Phylum) in free *Blastocystis*. • INC of Clostridia, IHU_PC_PC_Bacteria, Elusimicrobia, Lentisphaeria, Metanobacteria, and Deltaproteobacteria (Class) in Blastocystis carrier; INC Planctomycetacia, Rubrobacteria, Deinococci, Gammaproteobacteria, Actinobacteria, unassigned bacteriaand Bacilli (Class) in free *Blastocystis* sp. • INC Clostridiales, IHU_PO_Bacteria, Victivallales, Methanobacteriales, Elusimicrobiales, Aeromonadales, Acidaminococcales, and Desulfovibrionales in *Blastocystis* carrier (Order); INC in Planctomycetales, Rhodobacterales, Sphingomonadales, Rubrobacterales, Veillonellales, Pasteurellales, Micrococcales, Pseudonocardiales, Enterobacteriales, Myxococcales, Bifidobacteriales, unassigned bacteria, and Lactobacillales (Order) in free *Blastocystis*. • INC Clostridiaceae, Ruminococcaceae and Lachnospiraceae in *Blastocystis* carrier (Family); INC in Streptococcaceae, Bifidobacteriaceae, Enterobacteriaceaeand Leuconostocaceae in free *Blastocystis*. • INC in *Ruminococcus* and *Clostridium* (Genus) in *Blastocystis* carrier; INC *Streptococcus, Bifidobacterium* and *Shigella* (Genus) in free *Blastocystis*. • INC in *Clostridium saudii*, *Methanobrevibacter smithii*, *Faecalibacterium prausnitzii*, *Roseburia* sp. (Species) in *Blastocystis* carrier; INC *Streptococcus* sp., *Bifidobacterium* sp., *Shigella* sp. (Species) in free *Blastocystis*.	Eubiosis	(Kodio *et al.*, 2019)

***Blastocystis* (ST1-8)**	Amplicon-based NGS	NA	Principal Coordinate Analysis (PCoA) of Unweighted UniFrac distances, Principal Coordinate Analysis (PCoA) of weighted UniFrac distances.	• There were no significant differences between the IBS-*Blastocystis* sp. postive, IBS-*Blastocystis* sp.negative, healthy control-*Blastocystis* sp.positive and healthy control-*Blastocyctis* negative. • INC in Firmicutes (Phylum) in the IBS group compared to healthy control.	Dysbiosis	(Nagel *et al.*, 2016)
***Blastocystis* (ST2-ST3)**	Amplicon-based NGS	Chao1, Shannon indices	Principal component analysis (PCoA)	INC bacterial diversity in *Blastocystis* positive of assymptomatic individuals. • INC *Prevotella copri, Prevotella stercorea, Ruminoccoccus bromii, Alistipes putredinis, Bacteroides species, Bifidobacterium longum*, and *Oscillospira* sp. (Species); INC *Debaryomyces hansenii*, *Mucor mucedo*, *Aspergillus flavus*, *Mucor racemosus*, and *Issatchenkia terricola* (Species); DEC in *Hymenolepis nana* (Species).	Dysbiosis	(Nieves-Ramirez *et al.*, 2018)
***Blastocystis* (ST1-4,7,8)**	Amplicon-based NGS	Species richness, shannon diversity index	Bray-Curtis dissimilarities, Principal component analysis (PCoA)	• INC bacterial diversity and richness • DEC prevalent in *Bacteroides* enterotyped samples (Genus); INC in Methanobrevibacter relative abundances (Genus); ST4 being more prevalent in Ruminococcaceae entrotyped samples and associated with *Akkermansia* (Genus); ST2 was more prevalent in Ruminococcaceae entrotyped samples (Genus); ST3 inverse with *Akkermansia* (Genus).	Eubiosis	(Tito *et al.*, 2019)
***Blastocystis* sp.**	Amplicon-based NGS	Chao 1, species richness, Simpson	Bray-Curtis dissimilarities, Principal	• INC in Firmicutes, Bacteroidetes and Proteobacteria all patients' faecal samples (Phylum). • INC in Clostridia and Mollicutes in colonised patients (Class);	Eubiosis	(Audebert *et al.*, 2016)

		diversity indexes	Coordinate Analysis (PCoA) of Unweighted UniFrac distances	DEC in Bacilli (Class). • INC in Clostridiales, Erysipelotrichales Burkholderiales (Order). DEC in Lactobacillales, Bacteroidales (Order). • INC in Ruminococcaceae and Prevotellaceae (Family); INC Enterococcaceae, Streptococcaceae, Lactobacillaceae and Enterobacteriaceae (Family) in free- *Blastocystis*. • INC in *Prevotella*, *Acetanaerobacterium, Acetivibrio, Coprococcus, Hespellia, Oscillibacter, Papillibacter, Sporobacter*, *Ruminococcus, Roseburia* and *Faecalibacterium* (Genus).		
***Blastocystis* (ST 7)**	Real time PCR		NA	• DEC in *Lactobacillus* and *Bifidobacterium* in infected mices.	Dysbiosis	(Yason *et al.*, 2019)
***Blastocystis* (ST4)**	Amplicon-based NGS	NA	Principal component analysis (PCoA)	• INC bacterial richness in chronically infected rats. • INC in Proteobacteria and Tenericutes in infected animals (Phylum). • DEC in Firmicutes (phylum), *Bacteroideted*, *Clostridium, Pseudomonas and Rhodoplane (Genus); INC Anaerovorax, Oscillospira* and *Parabacteroides* (Genus)	Dysbiosis	(Defaye *et al.*, 2020)
Blastocystis	Real-time PCR		NA	• Has significant association between the presence of *Blastocystis* and *Clostridiodes difficile* infection.	Dysbiosis	(Vega *et al.*, 2020)
***Blastocystis* (ST1-4, ST6)**	Metagenomics		NA	• INC bacterial richness, *Blastocystis* mainly presence in individuals with *Prevotella* and *Ruminococcus* enterotypes (Genus)	Eubiosis	(Andersen *et al.*, 2015)
Blastocystis	Real-time PCR		NA	• INC in *Prevotella* (Species); DEC of *Bacteroides* (Genus) and *Clostridial* cluster XIVa (Species)	Eubiosis	(Andersen *et al.*, 2016)
***Blastocystis* (ST1-ST5)**	Real-time PCR		NA	• INC in *Bacteroides* sp. in IBS-C; DEC in *Bifidobacterium* sp., *Desulfovibrio* sp., *C. leptum* and *F. prausnitzii* (Species)	Dysbiosis	(Nourrisson *et al.*, 2014)

NA: Not available; INC: Increase; DEC: Decrease; NGS: Next-Gene Sequencing