**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 (Ajampur & 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 (Ajampur & 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.

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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 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 Blastocystis 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, leukemia, 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 phylogenetic 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; Beironvand 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 lifestyle 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 been 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; Asghhari 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 lamblia* 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, haemolyisis, irritable bowel disease (IBD), post cardiotomy patient, post traumatic splenectomised and dengue (Table 1). Several studies demonstrated higher infection of *Blastocystis* sp. were detected in patients under haemodialysis and post traumatic splenectomised as compared to healthy individuals (Karatasartova 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 ambiguous 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). Notably, 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, Ruminococcus 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-
Ramírez 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 Oscillospirawere significantly correlated with colonic hypersensitivity. A decrease in the overall content of SCFAs in the faeces 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 Bacteroidetes (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 (Rivièr 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 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 bacteria 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 dybiosis 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 dybiosis, 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 Bacteroidetes instead of those of the phylum Proteobacteria. Higher bacterial diversity of faecal microbiota comes from Blastocystis sp. colonised individuals rather than Blastocystis-free individuals. There were higher abundance of Clostridia class, Ruminococcaceae and Provotellaceafamilies in Blastocystis infected individuals while Enterobacteriacea 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 Bacteroidiaceae and Veillonellaceae 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 diversity of Ruminococcaceae enterotyped 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 Blastocystis with the higher specification 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 differentiation 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.

VI. REFERENCES

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VI. ACKNOWLEDGEMENTS

This study was fully funded by Ministry of Higher Education of Malaysia under the Fundamental Research Grant Scheme [USIM/FRGS/FPSK/055002/51119].
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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                | (Peña *et al.*, 2020) |
|                              | Iran       | Case control | 200                   | 15%     | 6%                | (Shafiei *et al.*, 2020) |
|                              | Indonesia  | Cross sectional | 454                   | 36.5%   | 28.6%             | (Kesuma *et al.*, 2019) |
|                              | Iran       | Case control | 282                   | 1.3%    | 12.3%             | (Beiromvand *et al.*, 2017) |
|                              | Jordan     | Case control | 209                   | 25.7%   | 9%                | (Jadallah *et al.*, 2017) |
|                              | France     | Case control | 112                   | 23.2%   | 16.1%             | (Nourrisson *et al.*, 2014) |
|                              | Mexico     | Case control | 100                   | 49%     | 48%               | (Vargas-Sanchez *et al.*, 2015) |
|                              | Denmark    | Case control | 483                   | 15%     | 22%               | (Krogsgaard *et al.*, 2015) |
|                              | India      | Case control | 250                   | 33.3%   | 15%               | (Das *et al.*, 2016) |
|                              | Thailand   | Case control | 126                   | 16.7%   | 10.0%             | (Surangsrirat *et al.*, 2010) |
|                              | Pakistan   | Case control | 315                   | 70%     | 29%               | (Yakoob *et al.*, 2010) |
|                              | Mexico     | Case control | 324                   | 15.7%   | 12.0%             | (Ramirez-Miranda *et al.*, 2010) |
| IBS, IBD & acute diarrhoea   | Rome       | Cross sectional | 2524                   | 22.3%   | 6.8%              | (Gabrielli *et al.*, 2020) |
|                              | Turkey     | Case control | 2334                  | 5.74%   | 3.12%             | (Cekin *et al.*, 2012) |
| HIV/AIDS                     | Ghana      | Case control | 192                   | 6.6%    | 20%               | (Cristanziano *et al.*, 2019) |
|                              | China      | Cross sectional | 311                   | 3.86%   | NA                | (Zhang *et al.*, 2019) |
|                              | Rome       | Cross sectional | 156                   | 25%     | NA                | (Fontanelli Sulekova *et al.*, 2012) |
| Country         | Study Type       | Sample Size | Prevalence | Follow-up Method | Reference                      |
|-----------------|------------------|-------------|------------|------------------|--------------------------------|
| Indonesia       | Cross sectional  | 52          | 21.8%      | Microscopy       | (Darlan et al., 2020)          |
| Iran            | Cross sectional  | 268         | 12.3%      | NA               | (Piranshahi et al., 2018)      |
| Iran            | Case control     | 140         | 15.0%      | Microscopy       | (Rasti et al., 2017)           |
| Nepal           | Cross sectional  | 112         | 2.2%       | NA               | (Ghimire et al., 2016)         |
| Burkina Faso    | Cross sectional  | 291         | 1.0%       | Microscopy       | (Sangaré et al., 2015)         |
| Iran            | Cross sectional  | 200         | 10.9%      | NA               | (Adarvishi et al., 2016)       |
| Laos            | Cross sectional  | 137         | 26.3%      | NA               | (Paboriboune et al., 2014)     |
| Indonesia       | Cross sectional  | 42          | 96%        | NA               | Culture (Idris et al., 2010)   |
| Iran            | Cross sectional  | 190         | 22.3%      | NA               | PCR (Esteghamati et al., 2019) |
| Iran            | Cross sectional  | 200         | 6.5%       | Microscopy       | Culture (Asghari et al., 2020) |
| Turkey          | Cross sectional  | 232         | 6.5%       | NA               | PCR (Yersal et al., 2016)      |
| China           | Cross sectional  | 381         | 7.1%       | NA               | PCR (Zhang et al., 2017)       |
| Iran            | Case control     | 122         | 3.3%       | NA               | Microscopy (Rasti et al., 2017)|
| Iraq            | Cross sectional  | 419         | 35.48%     | Microscopy       | (Ali et al., 2018)             |
| Iran            | Case control     | 236         | 9.3%       | Microscopy       | (Mohltashamipour et al., 2015) |
| Cameroon        | Cross sectional  | 235         | 2.7%       | Microscopy       | (Tangi et al., 2016)           |
| Iran            | Cross sectional  | 500         | 2.4%       | Microscopy       | (Fattahi Baghi et al., 2015)   |
| Iran            | Case control     | 501         | 9.1%       | Microscopy       | (Poorkhosravani et al., 2019)  |
| Egypt           | Cross sectional  | 185         | 20%        | NA               | Microscopy (Esmail et al., 2019)|
| Qatar           | Cross sectional  | 580         | 4.7%       | PCR              | (Boughattas et al., 2017)      |
| Netherland      | Case control     | 168         | 13.3%      | Microscopy       | (Rossen et al., 2015)          |
| Denmark         | Retrospective    | 316         | 14.9%      | PCR              | (Andersen et al., 2015)        |
| Location         | Study Type     | Sample Size | Positive Rate | Negative Rate | Methodology                    | Reference                                |
|------------------|----------------|-------------|---------------|---------------|--------------------------------|------------------------------------------|
| Belgium          | Cohort Study   | 1168        | 4%            | 30%           | PCR                           | (Tito et al., 2019)                      |
| Turkey           | Case Control   | 60          | 30%           | 13%           | Microscopy                    | (Karasartova et al., 2016)              |
| Egypt            | Case Control   | 101         | 54.5%         | 67.4%         | Microscopy                    | (Eassa et al., 2016)                    |
| Poland, Iran     | 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          | 16.25%        | NA            | Microscopy & PCR              | (Esteghamati et al., 2019)              |
| Turkey           | Cross sectional| 62          | 9.6%          | NA            | Microscopy                    | (Caner et al., 2020)                    |
| Brazil           | Retrospective  | 150         | 16%           | NA            | PCR                           | (Silva et al., 2020)                    |
| Brazil, Taiwan   | Case Report    | 1           | 100%          | NA            | Microscopy & PCR              | (Santos et al. 2014)                    |
| Iran             | Case Control   | 155         | 4.4%          | 0%            | Microscopy                    | (Rasti et al., 2017)                    |
| Brazil           | Cross sectional| 196         | 24.5%         | 41.9%         | Microscopy                    | (Gil et al., 2013)                      |
| China            | Cross sectional| 775         | 6.2%          | 7.6%          | Microscopy                    | (Li et al., 2014)                       |
| 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                  |
|-----------------------------------------------|------------|-----------------------------------------------|-----------------------------|
| **Inflammatory bowel syndrome (IBS)**         |            |                                               |                             |
| Chan                                          | NA         | NA                                            | (Peña et al., 2020)         |
| Indonesia                                     | 10.6%      | 1.5%                                          | (Kesuma et al., 2019)       |
| Iran                                          | 3.7%       | 11.1%                                         | (Beiromvand et al., 2017)   |
| Mexico                                        | 41%        | 24%                                           | (Vargas-Sanchez et al., 2015) |
| France                                        | 1.78%      | 3.57%                                         | (Nourrisson et al., 2014)   |
| Pakistan                                      | 86%        | 60%                                           | (Yakoob et al., 2010)       |
| Egypt                                         | 29.4%      | 43.14%                                        | (Fouad et al., 2011)        |
| India                                         | 6%         | 94%                                           | (Das et al., 2016)          |
| **Transplant**                                |            |                                               |                             |
| Brazil                                        | 37.5%      | 12.5%                                         | (Silva et al., 2020)        |
| Ghana                                         | 19%        | 9.5%                                          | (Cristanziano et al., 2019) |
| Rome                                          | 30.8%      | 7.7%                                          | (Fontanelli Sulekova et al., 2019) |
| Iran                                          | 21.6%      | 11.8%                                         | (Piranshahi et al., 2018)   |
| Turkey                                        | 10%        | 10%                                           | (Karasartova et al., 2016)  |
| **Cancer**                                    |            |                                               |                             |
| Turkey                                        | 23%        | 18%                                           | (Yersal et al., 2016)       |
| China                                         | 66.7%      | 59%                                           | (Zhang et al., 2017)        |
| Iran                                          | 20.9%      | 33.3%                                         | (Asghari et al., 2020)      |
| **Immunocompromised patients (IBS, IBD, acute diarrhoea)** | Rome | 29%                                           | (Gabrielli et al., 2020)    |
| Denmark                                       | 2.99%      | 1.49%                                         | (Andersen et al., 2015)     |
| Malaysia                                      | 33.3%      | 62%                                           | (Thergarajan et al., 2019)  |
| **Ulcerative colitis (UC)**                   |            |                                               |                             |
| **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 |
|------------|-----------------------------------------------|------------|
|            | ST1 | ST2 | ST3 | ST4 | ST6 | ST7 | Mixed |
| Chile      | NA  | NA  | -   | NA  | -   | -   | -     |
| Indonesia  | 7.1%| -   | 10% | -   | -   | -   | -     |
| Iran       | 7%  | 13% | 19% | -   | -   | -   | -     |
| Mexico     | 21% | 23% | 54% | -   | -   | -   | -     |
| France     | -   | -   | 8%  | -   | -   | -   | -     |
| Pakistan   | 86% | 60% | 47% | 75% | -   | -   | 50%   |
| Egypt      | -   | 26.53% | 34.7% | 30.61% | - | - | - |
| India      | 3%  | -   | 12% | -   | -   | -   | -     |
| Ghana      | 47.6% | 9.5% | 4.76% | - | - | - | - |
| Denmark    | 2.54% | 4.24% | 4.24% | 7.20% | 2.12% | - | - |

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 α-diversity | β-diversity | Gut microbiota composition shift | Status | References |
|----------|--------|---------------------------------|-------------|---------------------------------|--------|------------|
| *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 Eubiosis (Gabrielli et al., 2020) | 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 *Veillonelaceae* (Family). | INC in *Prevotella*, *Methanobrevibacter*, *Ruminococus* (Genus) in |
Blastocystis carrier; INC in *Bacteroides*(Genus) in free *Blastocystis* sp.

| B. *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, Ruminococaceae and Lachnospiraceae in *Blastocystis* carrier (Family); INC in Streptococaceae, 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. positive, IBS-Blastocystis sp. negative, healthy control-Blastocystis sp. positive and healthy control-Blastocystis negative. |
|                         |                   |    | • INC in Firmicutes (Phylum) in the IBS group compared to healthy control. |

| **Blastocystis** (ST2-ST3) | Amplicon-based NGS | Chao1, Shannon indices | Principal component analysis (PCoA) |
|----------------------------|--------------------|-----------------------|-----------------------------------|
|                            |                    |                       | INC bacterial diversity in *Blastocystis* positive of asymptomatic individuals. |
|                            |                    |                       | • INC *Prevotella copri*, *Prevotella stercorae*, *Ruminococcus 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). |

| **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 enterotyped samples and associated with *Akkermansia* (Genus); ST2 was more prevalent in Ruminococcaceae enterotyped samples (Genus); ST3 inverse with *Akkermansia* (Genus). |

| **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); |
| Blastocystis (ST 7) | Real-time PCR | NA | DEC in *Lactobacillus* and *Bifidobacterium* in infected mice. | Dysbiosis (Yason *et al.*, 2019) |
|---------------------|----------------|-----|-------------------------------------------------------------|----------------------------------|
| Blastocystis (ST4)  | Amplicon-based NGS | NA | • INC bacterial richness in chronically infected rats.  
• INC in Proteobacteria and Tenericutes in infected animals (Phylum).  
• DEC in Firmicutes (phylum), *Bacteroidetes*, *Clostridium*, *Pseudomonas* and *Rhodoplanes* (Genus); INC *Anaerovorax*, *Oscillospira* and *Parabacteroides* (Genus) | Dysbiosis (Defaye *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 (ST1-ST5) | 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., *Desulfovibrion* sp., *C. leptum* and *F. prausnitzii* (Species) | Dysbiosis (Nourrisson *et al.*, 2014) |

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