Original Article

Genetic characterization of *Blastocystis* spp. in patients with colorectal cancer, irritable bowel syndrome, and asymptomatic individuals in Sohag, Egypt

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ABSTRACT

Background: *Blastocystis* spp. is a common protozoan parasite that shows extensive genetic diversity with 16 different subtypes (STs) identified in humans.
Objective: To determine the detection rates and subtypes of *Blastocystis* spp. in colorectal cancer (CRC) patients, irritable bowel syndrome (IBS) patients, and asymptomatic individuals (participants with no GIT symptoms) in Sohag, Egypt.
Subjects and Methods: The study was conducted on 225 stool samples collected from 75 CRC patients, 75 IBS patients, and 75 asymptomatic participants with no GIT symptoms. All samples were examined microscopically by direct smears and formalin ethyl acetate concentration technique (FECT), and cultured on modified Jones' medium supplemented with 10% donkey serum. The genomic DNA was extracted from positive stool samples. Conventional and nested PCRs (nPCR) were used to amplify the small-subunit ribosomal RNA (SSU rRNA) encoding gene of *Blastocystis* spp.; and subtypes were identified by restriction fragment length polymorphism (RFLP) of nested PCR products using *Hinf* I and *Rsa* I.
Results: Out of 225 stool samples, *Blastocystis* was detected in 97 samples (43.1%) by culture, 89 samples (39.6%) by direct smears, and 71 samples (31.6%) by FECT with significant difference (P=0.035). The sensitivity of direct smears and FECT compared to culture was 87.6% and 73.2% while the specificity was 96.9% and 100%, respectively. The presence of *Blastocystis* spp. was significantly higher in CRC (53.3%) and IBS groups (48%) than in the asymptomatic group (28%) (P=0.004). Out of 97 positive fecal samples, *Blastocystis* SSU rRNA gene was amplified in 45 (46.4%) samples by conventional PCR and 95 (97.9%) samples by nPCR. RFLP analysis identified three STs, where ST3 was more common in CRC than IBS and asymptomatic groups (85%, 74.3%, 75%, respectively) followed by ST1 (15%, 20%, 25%, respectively). While ST2 was recorded in only 5.7% of the IBS group. However, there was no significant difference between *Blastocystis* STs and socio-demographic, and clinical groups.
Conclusion: Detection rate of *Blastocystis* spp. was higher in CRC patients and IBS patients than in asymptomatic group and ST3 was the most prevalent subtype in studied groups.

Keywords: asymptomatic, *Blastocystis* spp., CRC, IBS, PCR-RFLP, subtyping.

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INTRODUCTION

Blastocystis is one of the ambiguous protozoa of medical and veterinary importance[1], with variable recorded prevalence in different parts of the world, 5%-23% in developed countries, and up to 60% in developing countries[2]. It colonizes digestive systems of multiple hosts, and is transmitted via the fecal-oral route from contaminated water, foods; some animals are considered the most probable means of spread[3]. *Blastocystis* spp. possess multiple evolutionary or life cycle stages: vacuolar, granular, multi-vacuolar, avacuolar, ameboid, and cystic forms are recognized[4]. The main methods to detect *Blastocystis* spp. are microscopy, culture, and molecular assays[5].

Clinical features of the disease are non-specific including acute or chronic diarrhea, and abdominal pain. However, the pathogenicity of *Blastocystis* spp. is controversial because most patients are asymptomatic, which prompted several studies to consider *Blastocystis* spp. a commensal micro-organism. In fact, symptoms may be similar to irritable bowel syndrome (IBS) or skin disorders such as urticarial or allergic lesions[6]. In addition, several epidemiological studies reported a high infection rate of *Blastocystis* in patients with IBS and in immunocompromised patients such as, patients with malignancies[7,8].

In fact, IBS is a chronic functional gastrointestinal disease presenting clinically by abdominal pain with alteration of bowel habits. Although IBS has uncertain etiology, chronic gut inflammation due to persistent exposure to an infectious agent including *Blastocystis* spp. was proposed[9]. On the other hand, CRC is the fourth most common and third most deadly diagnosed

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cancer in the world. Nevertheless, infectious agents could be responsible for 20% of cancer. Recent findings have indicated the association of *Blastocystis* to CRC and recommend routine screening for *Blastocystis*[10,11].

Blastocystis exhibits considerable genetic variety, with at least 40 distinct subtypes (STs 1-17, ST21, and STs 23-44) found in a wide range of hosts, including humans, rodents, birds, primates, and other animals. Only 16 subtypes (STs1-10, ST12, ST14, ST16, ST23, ST35 and ST41) were isolated from humans[12,13], with ST1–4 being the most frequent, representing around 90% of all human surveyed[14]. Therefore, the aim of this study was to identify the infection rate, and STs of *Blastocystis* spp. in different groups including CRC patients, IBS patients and asymptomatic individuals in Sohag, Egypt.

SUBJECTS AND METHODS

This observational analytical study was carried out during the period from October 2022 to September 2024 in the Department of Medical Parasitology, Faculty of Medicine, Sohag University, Egypt.

Study area: The research was conducted on samples from Sohag Governorate of Upper Egypt. Sohag is in the south of the country, 467 km south of Cairo. It covers an area of 1547km^2 of the Nile Valley with.

Study design: A total of 225 stool samples were collected from patients complaining of CRC, IBS, and asymptomatic participants with no gastrointestinal symptoms. All participants filled a structured questionnaire, which covered complaints and demographic and clinical data. Collected stool samples were examined by wet mounts, concentration technique, and *in vitro* culture. *Blastocystis* positive stool samples were subjected to PCR-RFLP for genotyping.

Study participants: The study recruited 75 CRC patients from outpatient clinic of General and Oncological Surgery, 75 IBS patients from outpatient clinic of Gastroenterology at Sohag University Hospitals. An additional 75 asymptomatic individuals who were volunteer participants with no GIT symptoms and came to be screened for parasites as part of routine check-up. Inclusion criteria included age groups (≥15 years old), and both genders The IBS patients were selected based on the Rome IV criteria[15]. Exclusion criteria were patients who had received anti-parasitic drugs 2 w prior to sample collection, patients with history of neoplasm other than CRC or had received oncological treatment.

Laboratory examination: Fresh stool samples were collected in clean, dry sealed labelled plastic containers. All samples were examined by direct wet smears, formalin ethyl acetate concentration technique (FECT), modified Kinyoun's acid-fast stain[16]. and *in vitro* culture on modified Jones' medium supplemented with 10% donkey serum[17]. Cultured tubes were incubated for 48-72 h at 37˚C, and were examined microscopically for the detection of *Blastocystis* spp. If there were no organisms found, the cultured tubes were checked every 48 h for up to 10 d, before considering the cultures negative.

Extraction of DNA: The genomic DNA was extracted from *Blastocystis* positive fecal samples confirmed by culture using QIAamp® DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The eluted DNA was stored at -20˚C until PCR analysis[18]. The extracted DNA was amplified using conventional and nested PCR (nPCR).

Conventional PCR[19]: A 600 bp fragment of the *Blastocystis* SSU-rRNA encoding gene was amplified using forward primer (RD5) 5'-ATCTGGTTGATCCTGCCAGT-3' and reverse primer (BhRDr)-5' GAGCTTTTTAACTGCAACAACG-3'. The PCR reactions were carried out in a total volume of 25 µl, including 12.5 µl of ABT 2X RED Mix kit (Applied biotechnology, Egypt), 1 µl of each primer of 20 pmol concentrations, 7.5 µl nuclease free water and 3 µl extracted DNA. The PCR reactions were conducted on Thermal Cycler (Thermo Scientific Finnzymes Arktik™ TCA4848) with an initial denaturation step at 95°C for 4 min, followed by 35 cycles as follows: denaturation at 95° C for 1min, annealing at 58° C for 1 min and, extension at 72° C for 2 min with a final extension step at 72°C for 10 min.

Nested PCR[8,20]: A 1.1 kbp region of *Blastocystis* SSU-rRNA encoding gene was amplified using two pairs of primers: Primer set A (SR1F and SR1R) for the first reaction and Primer set B for the second reaction. For the first reaction, amplifications were conducted as follows: an initial denaturation step at 94°C for 5 min and 35 cycles at 94°C for 1min (denaturation), 54°C for 5 min (annealing), 72°C for 1.5 min (extension) with a final extension step for 10 min at 72°C. For the second reaction, primer set B annealing temperature used was 49°C instead of 54°C. PCR Products were separated by electrophoresis on 1.5% agarose gel, stained by ethidium bromide (Promega) and then visualized under ultraviolet (UV) light. For each PCR set, negative control was included. A 100 bp DNA ladder was used as a size marker.

The RFLP analysis[20,21]: Amplified products of nPCR were digested with 2 restriction endonuclease enzymes; *Hinf* I and *Rsa* I (Thermo Fisher Scientific, USA) to differentiate subtypes of *Blastocystis*. The total volume of the reaction mix was composed of 25 µl containing 10 µl PCR product, 2.5 µl 10 X buffer, 1 µl restriction enzyme and 11.5 µl nuclease-free water. Restriction digestion was performed at 37˚C for one hour. The digested products were electrophoresed on

3% agarose gel stained with ethidium bromide and visualized with UV transilluminator. A 100 bp DNA ladder was used to estimate the sizes of the restriction fragments. Interpretation of the resulting products of digestion and identification of *Blastocystis* STs were performed by comparison to patterns described by Clark[22] and modified according to the consensus terminology previously described[23].

Statistical analysis: Results were gathered, organized and tabulated in an Excel 2016 spreadsheet. Data were analyzed using IBM SPSS Statistics for Windows version 25.0 and Medcalc version 15.8.0. Chi-square (X^2) test was used for comparison regarding qualitative variables. Sensitivity, Specificity, positive and negative predictive values (PPV, NPV) and accuracy were calculated to evaluate diagnostic methods considering *in vitro* culture as "gold standard". Cohen's kappa and its significance were calculated to assess the agreement between the diagnostic methods, with the level of significance set using the following criteria: ≤0= poor, 0.01–0.20= slight, 0.21–0.40= fair, 0.41–0.60= moderate, 0.61–0.80= substantial, and 0.81–1= almost perfect. Different variables; age, gender, residence and contact with animals were analyzed as predictors for the occurrence of *Blastocystis* spp. among study individuals using logistic regression. Significance was considered at $P<0.05$.

Ethical considerations: This study was conducted after approval of the Medical Research Ethical Committee (MREC) of Faculty of Medicine, Sohag University, Egypt with the IRB registration No. Soh-med-22-10-16. This study was registered at Clinical Trials.gov under registry No. (NCT05580393), and followed the tenets of the Declaration of Helsinki. Written informed consents were obtained from enrolled participants after explaining the procedure and the purpose of the study. Infected individuals were referred to a physician for treatment.

RESULTS

Overall, out of 225 stool samples submitted to the Parasitology laboratory during the study period, *Blastocystis* spp. was detected in 89 samples (39.6%) by direct smears and 71 samples (31.6%) by FECT compared to Modified Jones culture (97 samples) (43,1%) with significant difference (P=0.035) (Table 1). It occurred singly in 49 samples (50.5%) and in coinfection with other intestinal parasites in 48 samples (49.5%). The most frequent *Blastocystis* spp. coinfections were observed with *Cryptosporidium* spp. (22 cases), followed by *E. coli* (13 cases), *E. histolytica/ dispar* (12 cases), *G. lamblia* (8 cases), *C. cayetanensis* (1 case) and *H. nana* (1 case). The infection was significantly higher in CRC and IBS patients (53.3% and 48%, respectively) than asymptomatic participants (28%) (P=0.004) (Table 2, Fig 1). However, there was no statistically significant difference between the blastocystosis and age, gender, residence, or contact with animals (P=0.551, 0.644, 0.082 and 0.357, respectively) (Table 3).

The sensitivity, specificity, PPV, NPV and accuracy of direct smears compared to culture as gold standard were 87,6%, 96.9%, 95.5%, 91,2%, and 92.9%, respectively with almost perfect agreement (0.854) and those of FECT compared to culture were 73.2%, 100%, 100%, 83.1%, and 88.4%, respectively with substantial perfect agreement (0.757) (Tables 4, and 5).

Out of 97 positive fecal samples, *Blastocystis* SSU rRNA gene was amplified in 45 samples by conventional PCR with a sensitivity of 46.4%, while in 95 samples by nPCR the sensitivity was 97.9% (Figs. 2 and 3). RFLP analysis identified three subtypes, ST1 (18.9 %), ST2 (2.1%) and ST3 (78.9 %). There were no mixed subtypes. Among CRC group, ST3 was the most common (85%) followed by ST1 (15%). In the IBS group, ST3 was the

Table 1. Infection by *Blastocystis* spp. by culture, direct smears and FECT among the study participants.

	Culture	Direct smear	Concentration (FECT)	*P* value
Positive	97 (43.1%)	89 (39.6%)	71 (31.6%)	0.035*
Negative	128 (56.9%)	136 (60.4%)	154 (68.4%)	

***:** Significant ($P<0.05$).

Table 2. Infection by *Blastocystis* spp. among study groups by *in vitro* culture.

Asymptomatic (n=75)	IBS (n=75)	CRC (n=75)	Total (n=225)	*P* value
Positive	21 (28%)	36 (48%)	40 (53.3%)	0.004*
Negative	54 (72%)	39 (52%)	35 (46.7%)	

***:** Significant ($P<0.05$).

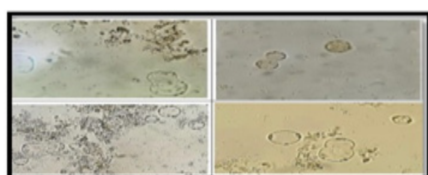

Fig. 1. Positive *Blastocystis* spp. samples showing binary fission of vacuolar forms detected in modified Jones' medium cultures (x1000).

Table 3. Infection by *Blastocystis* spp. according to socio-demographic criteria among the study participants.

Sociodemographic factors		*Blastocystis* spp. infection				*P* value
		Positive (n = 97)	Negative (n = 128)	Total (n = 225)	Crude OR (95% CI)	
Age	15-29 y	24 (24.7%)	37 (28.9%)	61 (27.1%)	0.8 (0.4-1.4)	0.551
	30-44 y	25 (25.8%)	26 (30.3%)	51(22.7%)	1.3 (0.7 -2.5)	
	45-59 y	25 (25.8%)	40 (31.3%)	65 (28.9%)	0.7 (0.4-1.3)	
	60-75 y	23 (23.7%)	25 (19.5%)	48 (21.3%)	1.2 (0.6-2.4)	
Gender	Male	50 (51.5%)	62 (48.4%)	112 (49.8%)	1.1(0.6-1.9)	0.644
	Female	47 (48.5%)	66 (51.6%)	113 (50.2%)		
Residence	Rural	62 (63.9%)	67 (52.3%)	129 (57.3%)	1.6(0.9-2.7)	0.082
	Urban	35 (36.1%)	61 (47.7%)	96 (42.7%)		
Animal Contact	Yes	56 (57.7%)	66 (51.6%)	122 (54.2%)	1.2(0.7-2.1)	0.357
	No	41(42.3%)	62 (48.4%)	103 (45.8%)		

Table 4. Efficacy of direct smears in comparison with modified Jones' culture as the "gold standard" in diagnosis of *Blastocystis* spp. infection.

Direct smear	Culture			Statistical analysis
	Positive	Negative	Total	
Positive	85	4	89	K (95% CI) = 0.854 (0.785-0.923) *P*<0.001*
Negative	12	124	136	
Total	97	128	225	
Sensitivity			87.6%	
Specificity			96.9%	
PPV			95.5%	
NPV			91.2%	
Accuracy			92.9%	

Table 5. Efficacy of FECT in comparison to modified Jones' culture as the "gold standard" in diagnosis of *Blastocystis* spp. infection.

Concentration (FECT)	Culture			Statistical analysis
	Positive	Negative	Total	
Positive	71	0	71	K (95% CI) = 0.757 (0.671-0.842) P <0.001*
Negative	26	128	154	
Total	97	128	225	
Sensitivity			73.2%	
Specificity			100%	
PPV			100%	
NPV			83.1%	
Accuracy			88.4%	

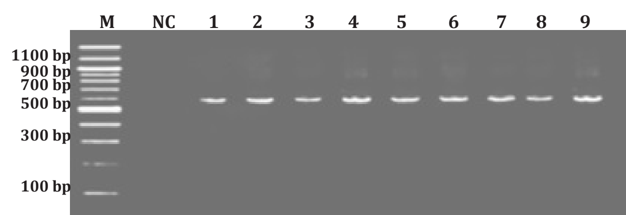

Fig. 2. Gel electrophoresis showing results of conventional PCR amplification of *Blastocystis* SSU-rRNA from positive fecal samples. **Lane M:** Molecular weight marker (100-bp DNA ladder); **Lane NC:** Negative control; **Lanes (1-9):** Positive samples showing bands at ~ 600 bp.

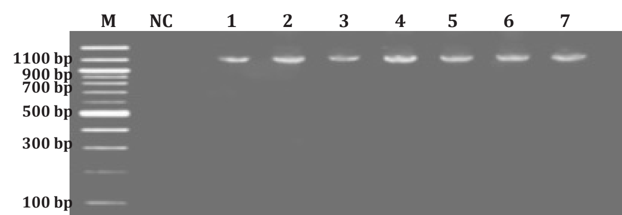

Fig. 3. Gel electrophoresis showing results of nPCR amplification of *Blastocystis* SSU-rRNA from positive fecal samples. **Lane M:** Molecular weight marker (100-bp DNA ladder); **NC:** Negative control; **Lanes (1-7):** Positive samples showing distinct bands at 1100 bp.

most common (74.3%) followed by ST1 (20%), and ST2 (5.7%). In asymptomatic group, ST3 was the most common (75%) followed by ST1 (25%) Also, there was no significant difference between *Blastocystis* STs and sociodemographic and clinical groups of participants (Table 6, Fig. 4).

Table 6. Infection by *Blastocystis* spp. according to socio-demographic criteria among the study participants.

Sociodemographic factors		*Blastocystis* subtypes			*P* value
		ST1 (n=18) (18.9%)	**ST2 (n=2) (2.1%)**	**ST3 (n=75) (78.9%)**	
Age	**15-29 y**	3 (16.7%)	1 (50%)	19 (25.3%)	0.357
	30-44 y	5 (27.8%)	1 (50%)	19 (25.3%)	
	45-59 y	8 (44.4%)	0 (0%)	17 (22.7%)	
	60-75 y	2 (11.1%)	0 (0%)	20 (26.7%)	
Gender	**Male**	8 (44.4%)	0 (0%)	40 (53.3%)	0.280
	Female	10 (55.6%)	2 (100%)	35 (46.7%)	
Residence	**Rural**	13 (72.2%)	2 (100%)	45 (60%)	0.346
	Urban	5 (27.8%)	0 (0%)	30 (40%)	
Animal contact	**Yes**	10 (55.6%)	2 (100%)	44 (58.7%)	0.477
	No	8 (44.4%)	0 (0%)	31 (41.3%)	
Clinical groups	**CRC (n=40)**	6 (15%)	0 (0%)	34 (85%)	0.344
	IBS (n=35)	7 (20%)	2 (5.7%)	26 (74.3%)	
	Asymptomatic (n=20)	5 (25%)	0 (0%)	15 (75%)	

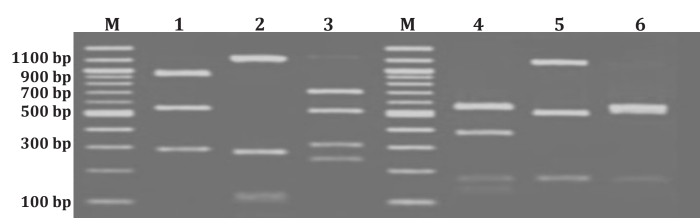

Fig. 4. Gel electrophoresis showing RFLP patterns of nPCR products of *Blastocystis* spp. after digestion with *Hinf* I and *Rsa* I. **Lane M:** Molecular weight marker (100-bp DNA ladder). The PCR products in lanes 1, 2 and 3 were digested with *Hinf I*, the PCR products in lanes 4, 5 and 6 were digested with *Rsa I*. **Lanes 1 and 4:** *Blastocystis* ST1; **Lanes 2 and 5:** *Blastocystis* ST2; **Lanes 3 and 6:** *Blastocystis* ST3.

DISCUSSION

Blastocystis spp. subtypes display genetic diversity. While some subtypes were associated with gastrointestinal symptoms and clinical manifestations, others were found in asymptomatic individuals, leading to debates about their pathogenic potential[24]. It was hypothesized that infection with *Blastocystis* spp. plays a role in the pathogenesis of CRC and IBS in humans[9,25]. In the present study, the significantly higher recorded infection by *Blastocystis* spp. in CRC patients (53.3%) and IBS patients (48%) than in asymptomatic participants (28%) (P=0.004), agreed with the results of three studies[18,21,26] that showed a significantly higher prevalence of *Blastocystis* spp. in symptomatic than asymptomatic participants (8.8% vs 2%[18], 39% vs 8%[21], and 58% vs 11%[26]). Two other studies[8,27] also found that detection of *Blastocystis* spp. was significantly higher in CRC patients than in the control group (12.15% vs 2.42%[8], and 29.7% vs 15%[27]).

As well, other studies[28-30] revealed that blastocystosis was significantly higher in IBS patients than in a control group (36.5% vs 28.6%, 57% vs 12%, and 46% vs 23%), respectively. However, our findings disagreed with results of Ali *et al*.[31] in Beni-Suef who found that there was no statistic difference in the infection rate with *Blastocystis* spp. between CRC patients 52% and control group 42%, and the results of Vargas-Sanchez *et al*.[32] who did not record statistical differences in the detection rates of *Blastocystis* spp. between IBS patients and the control group. The variations in the infection rates observed across different studies may be attributed to various factors such as geographical distribution, personal and community hygiene, cultural practices, climate changes, and the diagnostic techniques employed in each study.

The current study revealed that 50.5% of *Blastocystis* cases were singly infected by *Blastocystis,* and 49.5% showed poly-parasitic infection including *Cryptosporidium* spp., *E. histolytica/dispar*, and *G. lamblia*. These findings aligned with previous studies indicating that *Blastocystis* spp. is frequently associated with polyparasitism, particularly with intestinal protozoa. This polyparasitism may be attributed to sharing of gut parasites the same social conditions, environment, and the mode of transmission[7,33].

In the present study, the infection by *Blastocystis* was not significantly associated with age, sex, residence, or close contact with animal. These findings were consistent with the results of previous studies in which there was no statistical correlation between *Blastocystis* spp. infection and age, sex, residence or close contact with animal[8,12,21,31,34]. However, other

studies found that sociodemographic risk factors were associated with *Blastocystis* spp. infection[35,36].

Culture was considered the gold standard and revealed a higher detection rate of *Blastocystis* spp. compared to direct fecal smears and FECT and the sensitivity of direct examination and FECT compared to culture was 87.6% and 73.2% while the specificity was 96.9% and 100%, respectively. Our findings agreed with previous studies in which the *in vitro* culture was more sensitive than microscopic examination in detecting *Blastocystis* spp.[9,31,34,37,38]. *In vitro* culture was a simple, easy and sensitive method to detect *Blastocystis* spp. in stool specimens compared to microscopy. Low intensity of parasitic infection and intermittent shedding of gut parasites in stool specimens increased the number of false negative cases diagnosed by microscopic examination of wet mount smears[1,39].

In the current study, out of 97 positive samples, conventional PCR detected *Blastocystis* spp. in only 45 samples with a sensitivity of 46.4%, while nPCR detected *Blastocystis* spp. in 95 samples with a sensitivity of 97.9%. The findings aligned with previous studies[21,31,34,37], where culture was more reliable than molecular methods for *Blastocystis* detection and the sensitivities of conventional PCR were 57.5%, 85% 95% and 80%, respectively compared to *in vitro* culture. The findings disagreed with the results of Boutahar *et al*.[12] who considered conventional PCR the gold standard and revealed a higher detection rate of *Blastocystis* (67.62%) compared to culture in Jones' medium (51.43%), and direct examination (20.95%). The culture showed 76.1% sensitivity, 100% specificity while microscopy displayed 31% sensitivity and 100% specificity.

Discrepancies in PCR sensitivities observed across different studies can be attributed to several factors, such as the type of PCR (conventional, nested, real-time) and the type of samples utilized (feces or culture/fresh or frozen). PCR of DNA extracted from culture had higher sensitivity than PCR of DNA extracted from stool in detecting *Blastocystis* spp.[40]. The present study showed that ST3 (78.9 %) was the most common subtype, followed by ST1 (18.9 %) and then ST2 (2.1%) in all study participants. Our findings were in line with previous studies in Egypt conducted by El-Taweel *et al*.[21] in Egypt, where ST3 (55%) was found to be the predominant subtype followed by ST1 (20%), ST4 (15%) and ST2 (10%) in symptomatic and asymptomatic participants; and Ahmed *et al*.[41] who found that ST3 was the most common subtype (45.5%), followed by ST2 and ST1 (each 27.3%) in symptomatic and asymptomatic participants. Moreover, previous studies in Saudi Arabia and China reported nearly similar results stating that ST3 was the most common type followed by ST1 and ST2 in symptomatic and asymptomatic participants[17,26]. However, some studies as in Tanzania and China found that ST1 was more prevalent than ST3 in diarrheic and non-diarrheic outpatients[42,43]. While other studies revealed that ST2 was the predominant in Spain and Iran[44,45].

The current study showed that most infected CRC patients had ST3 (85%), while the remaining 15% were infected with ST1. The findings were similar to results of a study in Iran where ST3 was the most common subtype (37.5%), followed by ST2 (33.3%), ST1 (20.9%), and ST7 (8.3%) in cancer of children[46]. A study in Poland also reported the predominance of ST3 subtype in the CRC patients (75%), followed by ST1 (16.7%) and ST2 (8.3%)[8]. As well, another study from Egypt conceded that ST3 was the most prevalent (40%), followed by ST7 (30%), then ST2 (20%), and ST1 (10%) in CRC patients[31]. In contrast, a study from Saudi Arabia showed that ST1 was the predominant subtype in CRC patients (54.5%)[27]. Similarly, ST3 was the most common subtype (74.3%) among infected IBS patients, followed by ST1 (20%), then ST2 (5.7%). Our findings agreed with another study from Egypt indicating the predominance of ST3 subtype in IBS patients (81%), followed by ST1(19%)[9]. However, a study from Indonesia also concerned with ST1 subtype reported that it is the main one in IBS patients (10.9%) followed by ST3 (10.2%)[28]. Overall, 75% of asymptomatic carriers were found to be infected with ST3, while 25% were infected with ST1. This finding was in line with results of a study in Philippines in which ST3 was the most common subtype (65.5%), followed by ST1 (31.0%) and ST4 (3.44%) among asymptomatic carriers[47]. Notably, variability in distribution of *Blastocystis* subtypes from one country to another and within the same country depends on the prevailing epidemiologic situation and climatic conditions[48]. Subtype ST3 is more anthropogenic being the most frequently isolated subtype in humans, and is present in symptomatic and asymptomatic individuals[49].

We recorded no association between *Blastocystis* STs and sociodemographic and clinical groups of participants, a finding that agreed with two studies[46,50] that did not record significant difference between frequency of *Blastocystis* subtypes in symptomatic and asymptomatic cancer patients. Ali *et al*.[31] also found no variance in the distribution of STs in CRC patients and control group.

In conclusions, the detection rate of *Blastocystis* spp. infection in CRC patients and IBS patients was higher than in asymptomatic group. *In vitro* culture method had higher sensitivity, and specificity than direct smears and FECT in detecting *Blastocystis* spp. in fecal samples. The molecular testing revealed that nPCR was more sensitive than conventional PCR in detecting *Blastocystis* spp. ST3 as the predominant *Blastocystis* spp. in study groups. No association was found between *Blastocystis* STs and sociodemographic and clinical groups of participants. Routine stool microscopy along with *in vitro* culture could serve as

an alternative detection tool for the identification of *Blastocystis* in resource-poor diagnostic laboratory settings. Further studies are required to determine the role of *Blastocystis* spp. in the pathogenesis of IBS and CRC.

Author contribution: El-Hady A and Ahmed A designed the study. Abd Ellah A, and Mohamed S collected the data, conducted the laboratory investigations, and contributed to editing and revising the manuscript. All authors participated in revision of the manuscript, and approved the final version before publication.

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