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Blastocystis species prevalence and associated patient characteristics as predictors among a cohort of symptomatic and asymptomatic Egyptians

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ORIGINAL ARTICLE

Blastocystis Species Prevalence and Associated Patient Characteristics as Predictors Among a Cohort of Symptomatic and Asymptomatic Egyptians

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Abstract

Background: ***Blastocystis* spp. is a large unicellular intestinal protozoan parasite with a worldwide distribution. It has unclear pathogenicity and is linked to many clinical disorders. This study aimed to determine the prevalence of *Blastocystis* spp. molecularly in a cohort of symptomatic and asymptomatic individuals and to assess the association of *Blastocystis* spp. with the patient characteristics as possible predictors of blastocystosis.**

Methods: **Fecal specimens were collected and examined coproscopically for detection of gut parasites and cultured on Modified Jones' medium for detection of *Blastocystis* spp. Molecular assay using nested polymerase chain reactions (PCR) targeting *Blastocystis* copro-DNA was performed. The association between detection of *Blastocystis* spp. and patient demographics and clinical data was determined.**

Results: **Prevalence of parasitic infections was 62 (44.6%) using coproscopy. *Blastocystis* spp. was the prevalent parasite (21.6%), followed by *Entamoeba histolytica* complex (13.7%) and *Giardia intestinalis* (10.8%). *Cryptosporidium* spp. (2.2%) and *Entamoeba coli* (2.1%). Among the studied patient characteristics, only age showed statistical significance in association with detection of *Blastocysitis* spp. Microscopy for detection of *Blastocystis* was of perfect specificity but limited by false negatives (16.7%). All the positive cases by culture were confirmed positive by PCR.**

Conclusion: ***Blastocystis* remains a prevailing gut parasite in both symptomatic and asymptomatic studies in Egypt. Only gastrointestinal (GIT) symptoms showed significant correlation with detection of *Blastocystis* in stool, and can be a predictor of the probability of having blastocystosis. Further studies are required to speciate *Blastocystis* and to determine its role in health and disease.**

Keywords: *Blastocystis*, Culture, Microscopy, Polymerase chain reactions, Predictors

1. Introduction

Blastocystis spp. is an anaerobic unicellular enteric protozoan parasite of worldwide distribution, it is the most commonly isolated microorganism in human fecal samples.[1] *Blastocystis* spp. exists in stool or culture in many forms, including vacuolar, granular, amoeboid, and multivacuolar, a vacuolar, and cyst forms. Vacuolar cysts are the dominant form found in the environment (soil and water) that can transmit the parasite to humans.[2]

The infective form is the cyst stage, two types of cysts are formed: thin-walled cysts which contain schizonts and possibly cause autoinfection, whereas thick-walled cysts are responsible for the external fecal-oral route of transmission.[3,4]

There is controversy about the pathogenicity of *Blastocystis* spp. It was reported to be part of intestinal microbiomes in healthy individuals (eubiosis),[5] while other studies linked it to gut disorders (dysbiosis) and the induction of growth of rectum and colon cancer.[6] Blastocystosis has a wide range of

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clinical pictures, it may be asymptomatic, or present with nonspecific gastrointestinal (GIT) symptoms, including diarrhea, nausea, vomiting, flatulence, cramps, discomfort, abdominal pain, fever, urticaria, or anorexia.[7]

Blastocystis spp. has been detected worldwide, with up to 100% prevalence, and has a higher prevalence in developing countries (30–50%) than in developed countries (1.5–10%).[8] In Egypt, prevalence rates, up to 67.4% were reported in humans.[9,10] The high prevalence in developing countries is related to inadequate hygiene, close contact with animals, and consumption of contaminated food or water.[11,12]

Laboratory diagnosis is based on microscopic examination of stool specimens to detect *Blastocystis* cysts, which is easily recognized by its large size and characteristic appearance.[13] The variety of *Blastocystis* spp. morphologies in fecal specimens can however result in false positive results. The culture method usually used to confirm the diagnosis due to its higher sensitivity and specificity[14] but it is a time-consuming method. To overcome these limitations, several molecular polymerase chain reactions (PCR)-based diagnostic approaches using faeces directly or after culture of faecal specimens have been used.[15]

The purpose of the current study was to determine molecularly the prevalence of *Blastocystis* spp. in a cohort of symptomatic and asymptomatic individuals and to assess patient characteristics as a predictors for occurrence of *Blastocystis* spp.

2. Materials and methods

2.1. Study subjects

A hospital-based cross sectional study was carried out on faecal specimens from 139 individuals attending Cairo University Hospital clinics from June 2020 to October 2021, for screening for parasite as part of routine check-up (asymptomatic group) or have GIT symptoms (symptomatic group). Patients ranged in age from 5 months to 74 years (mean 31.7 ± 19.47); 78 (56.1%) were males while 61 (43.9%) were females. Demographic and clinical data of all participants were recorded.

2.2. Ethical approval and consent to participants

The research began after the study was approved by the ethical committee of the Faculty of Medicine at Al-Azhar University. All the parents/guardians of the child patients were informed verbally about the research purpose, and the collection of specimens was completed after obtaining their consent.

2.3. Collection of stool samples

Participants were asked to submit single fresh stool specimens free of water and urine. Stool specimens were collected in clean, dry, labelled plastic containers, sent immediately to the parasitology laboratory, and divided into three parts:

The first part was examined coproscopically using direct wet mount prior to and after concentration and permanent staining with modified Ziehl-Neelsen (ZN) stain and Wheatley's modified Trichrome stain for detection of gut parasites.[16]

The second part (about 50 mg) of all fresh stool specimens,[10] was cultured for *Blastocystis* by direct inoculation into culture tubes with 5 ml of Jones' medium enriched with horse serum (10%).[17] Cultured tubes were incubated for 48–72 h at 37 °C. The cultured tubes were examined microscopically for the detection of *Blastocystis* spp. after 48–72 h. If there were no organisms found, the cultured tubes were checked every 48 h until 10 days, before reporting negative cultures for *Blastocystis*.

The third part was kept fresh frozen and was used to extract DNA using QIAamp Fast DNA Stool Mini Kit (QIAGEN, Germany) according to the kit's instructions. The eluted DNA was stored at −20 °C for PCR assays. Extracted DNA was amplified using the primers: Reverse primer BhRDr (GAGCTTTTTAACTGCAACAACG) and Forward primer RD5 (ATCTGGTTGATCCTGCCAGT). PCR reaction and reaction conditions were performed as described previously (El-Badry et al., 2018).[11] Fragment of 550–585 basepairs of the amplified PCR products were separated on a 1.5% (w/v) agarose gel (Promega), stained with ethidium bromide (Promega), and visualized under UV light. Positive and negative controls were included for every PCR reaction.

2.4. Statistical analysis

Using the statistical package software SPSS model 26 (Chicago, IL, USA) data was tabulated, and the descriptive statistics for quantitative and qualitative variables were defined using mean, SD, frequency and percentage. Statistical significance was made using the χ^2 test, and data was considered statistically significant if the P value was less than 0.05. Diagnostic performance [specificity, positive predictive value (PPV), sensitivity and negative predictive value (NPV)], accuracy, and Kappa agreement of the diagnostic tests were all conducted. To identify *Blastocystis* predictors, all study population variables were entered into logistic regression models using ENTER method and

prediction was measured by odds ratio and considered significant if *P*-value <0.2.

3. Results

The study revealed an overall prevalence rate 44.6% (62/139) of parasitic infections of using direct wet mount smears. *Blastocystis* spp. was the most prevalent parasite (21.6%), followed by *Entamoeba histolytica* (*E. histolytica*) complex (13.7%), and *Giardia intestinalis* (*G.intestinalis*) (10.8%). *Cryptosporidium* spp. (2.2%) and *Entamoeba coli* (2.1) were the least detected parasites (Fig. 1 and Table 1). *Blastocystis* spp. was positive by light microscopy in 21.6% (30/139), by both culture and PCR in 25.9% (36/139) of cases. *Blastocystis* spp. showed confection in five cases, three cases with *E. coli*, one case with *E. histolytica* complex and one case with *G. intestinalis* (Table 2). All the positive cases by culture were confirmed positive by PCR (Fig. 2).

The sensitivity of microscopy was 83.3% (CI:0.62–0.94), specificity 100% (CI:0.93–1), PPV 100% (CI:0.80–1), NPV 94.5% (CI:0.86–0.98), accuracy 95.7% (CI:0.89–0.99) with almost perfect agreement (88%) versus yield of culture as a gold reference test (Table 3).

Age was statistically significantly associated with *Blastocystis* infection, middle-aged adults were the highest age-group infected by *Blastocystis* (10.8%), followed by school children (7.2%). *Blastocystis* spp. was more prevalent in males (14.4%) than females (10.5%) with no statistical significance. *Blastocystis* spp. infections were more prevalent in individuals living in rural areas (15.8%) than in urban settings (10.1%), and in symptomatic (16.5%) than in asymptomatic (9.4%) people, with nonstatistical significance (Table 4). Patients presented a nonspecific GIT symptom (diarrhoea, flatulence, vomiting, abdominal pain, and nausea).

Individual characteristics (variables): age group, sex, residency, and symptomatic/asymptomatic clinical status were analyzed as predictors for the occurrence of *Blastocystis* spp. among study individuals using logistic regression. Only GIT symptoms showed significant correlation with probability of having blastocystosis (*P* value = 0.13) (Table 5).

Blastocystis spp. among study population was 30 (21.6%) and 36 (25.9%) by microscopy and culture respectively. All cases positive by culture were confirmed positive by PCR.

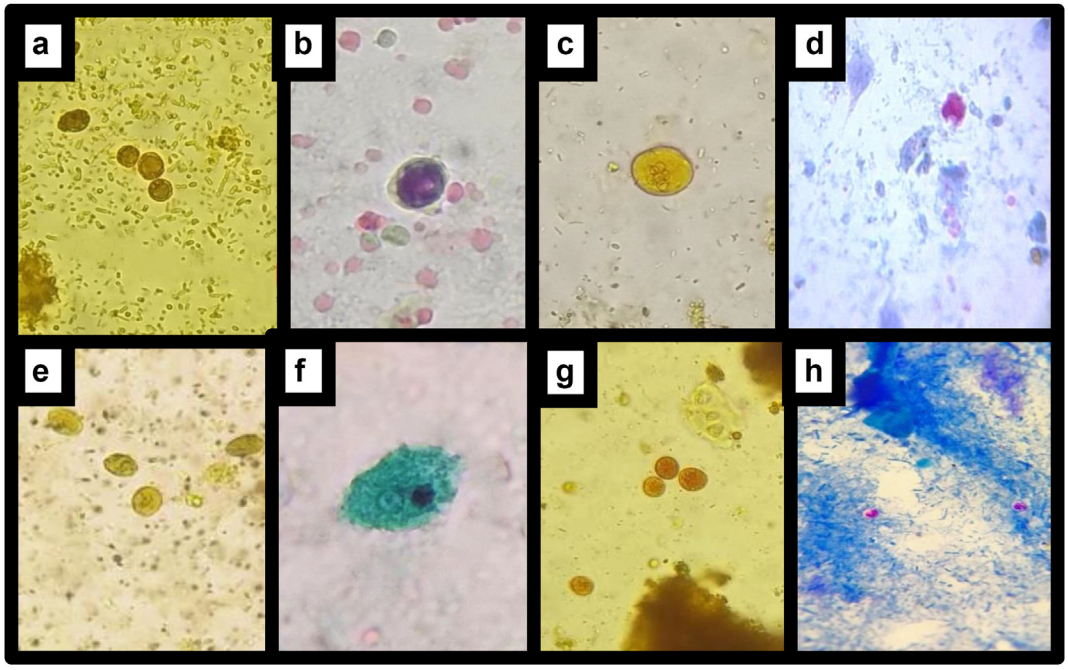

Fig. 1. a-Blastocystis *spp. vacuolar form from a stool sample; central vacuole with a thin peripheral rim of cytoplasm and multiple nuclei (Iodine stain). b-Vacuolar forms of* Blastocystis *spp. (Trichrome stain). c*-E. coli *cyst (Iodine stain). d- Giardia intestinalis trophozoite (Trichrome stain). e*-Giardia intestinalis *cyst (Iodine stain). f*-E. histolytica *trophozoite (Trichrome stain). g*-E. histolytica *cyst (Iodine stain). h*-Cryptosporidium *spp. oocyst (modified ZN stain).*

Table 1. Results of microscopy for detection of prevailing parasites.

			Frequency	Percent
Microscopic examination	Parasites	*Blastocystis* spp.	25	18.0
		E.histolytica complex	15	10.8
		G. intestinalis	11	7.9
		Cryptosporidium spp.	3	2.2
		Blastocystis spp. and *E.histolytica* complex	1	0.7
		Blastocystis spp. and *E.coli*	3	2.2
		Blastocystis spp. and *G.intestinalis*	1	0.7
		E.histolytica complex and *Giardia intestinalis*	3	1.4
		Total	62	44.6
	No parasites		77	55.4
Total			139	100.0

Table 2. Diagnosis, Prevalence of Blastocystis *spp. among study populations and the diagnostic performance of microscopy, culture and PCR assay.*

				Blastocystis spp. (microscopy)		
				+ve	−ve	Total
Blastocytis spp. Culture (and PCR)	Positive	Single infection		25 (18.0)	4 (2.9)	29 (20.9)
		Multiple infection	*Blastocystis* spp. and *E.histolytica* complex	1 (0.72)	2 (1.4)	3 (2.12)
			Blastocystis spp. and *E.coli*	3 (2.16)	0	3 (2.16)
			Blastocystis spp. and *G.intestinalis*	1 (0.72)	0	1 (0.72)
			Total	5 (3.6)	2 (1.4)	7 (5.0)
		Total		30 (21.6)	6 (4.3)	36 (25.9)
	Negative			0	103 (74.1)	103 (74.1)
	Total			30 (21.6)	109 (78.4)	139 (100)

4. Discussion

Blastocystis spp. was the most prevalent gut parasite in one fifth of our study individuals (21.6%). *Blastocystis* spp. has been detected worldwide and in Egypt, with varying prevalence's. It was reported as the most prevalent gut parasite in many Egyptian and global studies.[8,18–22]

Despite the relatively high prevalence of gut parasites in our study population, all of them were protozoa and there was no case with helminthic infection. This gut protozoa predominance may be attributed to the implementation in Egypt of a large-scale mass deworming strategy by the World Health Organization (WHO). It also may be a limitation of

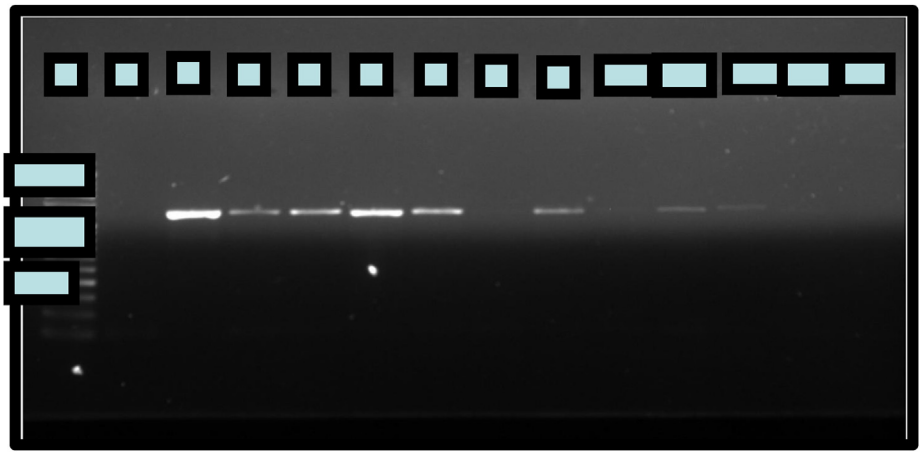

Fig. 2. The gel for PCR products targeting Blastocystis *ssu gene. Lane 1 L50 is molecular weight marker, 50 bp. lane 2 is negative control, Lane 3 is positive control, lanes (4–7), (9), (11,12): PCR positive samples showing distinct band at 600 bp; (8), (10), (13–14): PCR negative samples.*

Table 3. Diagnostic performance (sensitivity, specificity, PPV, NPV and accuracy) and Kappa agreement of microscopy considering culture as the gold standard.

	Microscopy (%, CI)
Sensitivity	83.3% (0.62–0.94)
Specificity	100% (0.93–1)
PPV	100% (0.80–1)
NPV	94.5% (0.86–0.98)
Accuracy	95.7% (0.89–0.99)
Kappa[a]	0.88 (0.67–1.09)

[a] **Key for Kappa:** < 0 Poor agreement. 0.01–0.20 Slight agreement. 0.21–0.40 Fair agreement. 0.41–0.60 Moderate agreement. 0.61–0.80 Substantial agreement. 0.81–1.00 Almost perfect agreement.

our one center study, small-scale study comprised of a small number of participants, and therefore it is difficult to precisely determine the real prevalence.

In the present study 18% of *Blastocystis* cases were singly infected by *Blastocystis* and 3.6% showed poly-parasitic infection. Only *Blastocystis* spp. (3.6%) showed poly-parasitic infection with *Entamoeba* spp. (4 cases) and *G. intestinalis* (one case). Similarly, Belkhair and colleagues[22] in Morocco and Steinmann and colleagues[23] in China reported that *Blastocystis* spp. was the most frequent gut protozoa associated with polyparasitism, mainly with gut amoebas. This polyparasitism may be attributed to sharing of gut parasites the same social conditions, environment, and the mode of transmission.

Consistent with our results, *in vitro* culture was a simple, easy and sensitive method to detect *Blastocystis* spp. in stool specimens compared with corposcopy. 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 faecal smears. However, in medical laboratories, direct microscopy still considered easy, rapid, convenient, and economic for diagnosis of *Blastocystis* Elsayed and colleagues[19] and Abdo and colleagues[21]

All our positive cases study for *Blastocystis* by culture were confirmed positive using PCR assay. Stool cultures for detection of *Blastocystis* spp. may miss some true positive infections as a result of the degeneration of *Blastocystis* in culture Tan.[4] Also, Elghareeb and colleagues[24] reported that *in vitro* stool culture for detection of *Blastocystis* spp. is not necessarily more sensitive than other diagnostic methods. Detection of *Blastocystis* DNA using PCR-based method is the most effective and favorable diagnostic method. It is widely used as a standard reference technique because of its high sensitivity and specificity as well as its ability to reveal the subtypes of *Blastocystis* spp. El-Badry and colleagues[10] and Roberts and colleagues.[15]

In the present study, there was a statistical significance association between age and detection of *Blastocystis* in stool, middle-aged adults were the most *Blastocystis* infected group (10.8%) followed by school children (7.2%). Similarly, El-Taweel and colleagues[25] found that 45% of individuals infected with *Blastocystis* spp. were 20–40 years old. The study in Libya, *Blastocystis* spp. was significantly higher in adults (>18 years) Abdulsalam and colleagues[26] and El Safadi and colleagues.[27] The study

Table 4. Demographic, environmental and clinical variables of study individuals among Blastocystis *spp.*

	Blastocystis culture			*P*-value
	Positive No. (%)	Negative No. (%)	Total No. (%)	
Age group				
Infant	0	3 (2.2%)	3 (2.2%)	0.013
Preschool child	4 (2.9%)	9 (6.5%)	13 (9.4%)	
School child	10 (7.2%)	9 (6.5%)	19 (13.7%)	
Adolescent	0	11 (7.9%)	11 (7.9%)	
Young adult	7 (5.0%)	27 (19.4%)	34 (24.5%)	
Middle-aged adult	15 (10.8%)	35 (25.2%)	50 (36.0%)	
Old adult	0	9 (6.5%)	9 (6.5%)	
Sex				
Females	16 (11.5%)	45 (32.4%)	61 (43.9%)	0.937
Males	20 (14.4%)	58 (41.7%)	78 (56.1%)	
Residence				
Rural	22 (15.8%)	72 (51.8%)	94 (67.6%)	0.332
Urban	14 (10.1%)	31 (22.3%)	45 (32.4%)	
GIT-Symptoms				
Asymptomatic	13 (9.4%)	49 (35.3%)	62 (44.6%)	0.234
Symptomatic	23 (16.5%)	54 (38.8%)	77 (55.4%)	
Total	36 (25.9%)	103 (74.1%)	139 (100%)	

Data presented as n (%), *P*-value is statistically significant at <0.05.

Table 5. Logistic regression analysis for Blastocystis *spp. positive cases.*

	Frequency			OR	95% CI	*P* value*
	+ve	−ve	%#			
Sex						
Male/	20	58	34.5	1.41	(0.18–11.2)	0.74
Female	16	45	35.6			
Age group						
Preschool children/	4	9	44.4	0.16	(0.00–0.00)	1.0
Infant	0	3	0			
School child/	10	9	111.1	0	(0.00–0.00)	0.99
Infant	0	3	0			
Adolescent/	0	11	0	0	(0.00–0.00)	1.0
Infant	0	3	0			
Young adult/	7	27	25.9	0	(0.00–0.00)	0.99
Infant	0	3	0.00			
Middle-aged adult/	15	35	42.9	0	(0.00–0.00)	0.99
Infant	0	3	0.00			
Old adult/	0	9	0	0.74	(0.00–0.00)	1.0
Infant	0	3	0			
Residence						
Rural/	22	72	30.6	0.71	(0.18–8.47)	0.79
Urban	14	31	45.2			
Clinical status						
Symptomatic/	23	54	42.6	0.15	(0.01–1.80)	0.13
Asymptomatic	13	49	26.5			
Total	36	103	34.9			

Data presented as n (%), +ve = positive, −ve = negative, (#) % of *Blastocystis* within the same group, (*) *P*- value < 0.2 is significant.

in France, Paulos and colleagues[28] found that the prevalence of *Blastocystis* spp. was significantly higher in 15–49 years old. The study in Spain, Segui and colleagues.[29] found that children were more commonly infected by *Blastocystis* spp. But, Taiyaba and colleagues.[30] revealed homogenous distribution of *Blastocystis* spp. infection among all age groups.

In our study, prevalence of *Blastocystis* spp. was higher in males (14.4%) compared with females (10.5%) with no statistical significance. Higher prevalence of *Blastocystis* in males than females was reported by many studies Hamdy and colleagues, Abdulsalam and colleagues and El Safadi and colleagues,[20,26,27] however, Dagci and colleagues[31] detected higher rate of *Blastocystis* infection among females than males. Meanwhile, Beyhan and colleagues[32] found that prevalence of *Blastocystis* spp. in males and females was approximately the same, with no statistical significance correlation. The higher prevalence of *Blastocystis* in adult males of the current study may be due to cultural habit, where middle-aged adult males engage in more outdoor activities than females.

In the current study, *Blastocystis* spp. infections was more prevalent in individuals living in rural areas (15.8%) than urban settings (10.1%), with no statistical significance. These findings agree with Hamdy and colleagues[20] which found that the prevalence of *Blastocystis* spp. infection in rural areas (68%) was higher than urban settings (32%) areas, with no statistical significance. This may be explained by that people living in rural areas have more contact with contaminated soil and animals, inadequate sanitation, and drinking from improper water sources.

The possibility of asymptomatic carriers of *Blastocystis* spp. has also been reported,[32–34] as a possible source of infection. In the present study, *Blastocystis* spp. detection rate was higher in symptomatic (16.5%) than asymptomatic (9.4%) individuals, with no statistical significance. Unlike, Paulos and colleagues[28] and Hamdy and colleagues[20] whom did not find any correlation between GIT symptoms and infection with *Blastocystis*. Whereas, Salvador and colleagues[33] and Mohamed and colleagues[34] found that 70.2% and 64% of *Blastocystis* infected patients had GIT symptoms.

In our study, the individual characteristics (age group, sex, residency, and symptomatic/asymptomatic clinical status) were analyzed as predictors for the occurrence of *Blastocystis* spp. among study individuals using logistic regression. Only GIT symptoms showed significant correlation with the probability of having blastocystosis.

4.1. Conclusions

Blastocystis is a prevailing gut parasite among the studied Egyptians. Our study found that microscopy

for detection of *Blastocystis* is of perfect specificity but is limited by false negatives (16.7%). All cases positive by culture were confirmed positive by PCR. Only having symptoms showed significant correlation with detection of *Blastocystis* in stool, and thus can serve as a predictor of the probability of having blastocystosis. Further studies are required to speciate *Blastocystis* and to determine its role in gut health and disease.

Conflicts of interest

Authors declare that there is no conflict of interest, no financial issues to be declared.

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