



Evaluating the Knowledge and Practice of Physicians Regarding *Blastocystis hominis*

Maryam Fasihi Karami ¹, Molouk Beiromvand ^{2,*}, Abdollah Rafiei², Bahman Cheraghian³ and Ehsan Beigzadeh⁴

¹Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

²Department of Parasitology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

³Department of Biostatistics and Epidemiology, Alimentary Tract Research Center, School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

⁴IT Center, School of Dental Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

*Corresponding author: Department of Parasitology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Email: beiromvandm@gmail.com

Received 2022 December 05; Revised 2023 February 19; Accepted 2023 February 21.

Abstract

Background: *Blastocystis hominis* is an enteric protozoan in humans and animals. This survey aimed to assess the knowledge and practice of physicians about *B. hominis*.

Methods: This study was performed on physicians working in Ahvaz County, using a convenience sampling method from January to June 2020. Data were collected through face-to-face interviews and an electronic questionnaire. Descriptive statistics, including frequencies and percentages, were calculated for all variables. P-values < 0.05 were considered significant.

Results: Our results showed that of the 352 physicians, 64.2% and 58.0% knew that *B. hominis* might cause abdominal pain and diarrhea, respectively, but their knowledge was poor about the possibility of extraintestinal clinical symptoms such as urticaria. Regarding the transmission route, 91.8% agreed that the fecal-oral route is the main mode of *B. hominis* transmission, but only 17.9% were aware of the role of host animals in its transmission. Furthermore, the physicians had poor knowledge of the need to treat symptomatic patients and resistance to metronidazole in some cases. More than two-thirds of the physicians (68%) had good practice treating infected immunocompromised patients but had poor practice treating symptomatic patients.

Conclusions: This study emphasizes improving physicians' knowledge of pathogenicity, clinical symptoms, and treating *B. hominis*. Since *B. hominis* is a common protozoan with pathogenic potential, we recommend training sessions for physicians to inform them of new findings about *B. hominis*.

Keywords: Knowledge, Practice, *Blastocystis hominis*, Physician, Iran

1. Background

Blastocystis hominis is a common enteric protozoan in humans and animals (1, 2). This intestinal protozoan has a worldwide distribution with a prevalence of more than 1 billion people worldwide (3). *Blastocystis hominis* is more common in developing countries (4). Since this eukaryotic microorganism is reported in asymptomatic and symptomatic individuals, its pathogenicity remains controversial (3). In symptomatic cases, *B. hominis* has been linked to clinical manifestations, such as diarrhea, abdominal pain, irritable bowel syndrome (IBS), irritable bowel disease (IBD), constipation, flatulence, and urticaria (5-7).

Molecular studies on the small subunit of the ribosomal RNA (SSU rRNA) gene led to identifying at least 17 subtypes (ST1-ST17). ST1-ST9 and ST12 have been reported in humans and animals (2, 8). Therefore, these subtypes may

be zoonotic and transmitted by contact with infected animals and/or via contaminated water with *B. hominis* cysts through the fecal-oral route (8). Direct smear examination and xenic culture are typical methods for diagnosing *B. hominis*. Since culture is a time-consuming technique, molecular methods like real-time quantitative PCR (qPCR) assays have been proposed to detect *B. hominis* in stool samples (9). *Blastocystis hominis* has vacuolar, granular, multivacuolar, avacuolar, amoeboid, and cyst forms (10). The small cyst stage, the transmitter form, may be difficult to diagnose by stool examination (1). One of the important issues with *B. hominis* is whether or not to treat infected individuals. The controversial pathogenicity of the protozoan has made treatment equivocal (11).

In Iran, in a systematic review and meta-analysis study, a total prevalence of 3% was reported for *B. hominis* (12). In a study by Khademvatan et al. in 2018, intestinal parasites

were assessed in stool samples from Ahvaz County, and *Blastocystis* was observed in 14.35% of the examined stool samples (13). In another study conducted in Ahvaz County on 618 human fecal samples, *Blastocystis* had a prevalence of 23.6%, and ST3 was the most commonly observed ST (14). Despite the controversial treatment, diagnosis, and pathogenicity of this common intestinal protozoan, no study has yet been conducted to assess the level of knowledge and practice of the Iranian medical community about this parasite.

2. Objectives

The present study evaluated the knowledge and practice of physicians in Ahvaz County to include, if necessary, new findings about *B. hominis* in retraining the medical community.

3. Methods

3.1. Study Design

This cross-sectional study was performed on physicians working in private and government hospitals in Ahvaz County using a convenience sampling method from January to June 2020. Ahvaz County, the capital of Khuzestan Province, with a population of about 1.3 million and 815 km², is located in southwestern Iran. The county has 19 hospitals.

3.2. Sample Size

Due to the lack of appropriate estimates for the desired values in the target population and to maximize the sample size with $P = 0.5$ and $d = 0.05$, the sample size was calculated at 375 physicians. Twenty-three physicians who had answered only one or two questions were excluded from the study. The number of participants from each hospital was proportional to the number of physicians in that hospital.

3.3. Inclusion Criterion

The inclusion criterion was physicians with a work experience of more than two years and being residents of Ahvaz County.

3.4. Questionnaire

The questionnaire's first section consisted of gender, age, occupation, educational status, and duration of work experience. The second section of the questionnaire included eight knowledge-based and four practice-based

questions. Content validity was assessed using an evaluation of the items by ten experts, and the validity results were acceptable. Besides, test-retest reliability was assessed by answering the items by 30 participants twice over three weeks. The calculated correlation coefficient was 0.76, which indicates acceptable reliability. The survey data was collected through a face-to-face interview at the beginning of the study and was continued with an electronic questionnaire due to the outbreak of Coronavirus disease 2019 (COVID-19).

3.5. Data Analysis

Statistical analyses were conducted using SPSS 16 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics, including frequencies and percentages, were calculated for all variables. P -values < 0.05 were considered significant.

3.6. Ethics Approval and Consent to Participate

The protocol of the present study was reviewed and approved by the Ethics Committee of the Student Research Committee, Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1398.836). The methods were carried out in accordance with relevant guidelines and regulations.

4. Results

4.1. Socio-demographic Characteristics

Of the 352 physicians, 187 (53.1%) were male and 165 (46.9%) were female. The average age of the participants was 40.1 (SD: 8.3; range = 25 - 59 years old). The physicians included 158 (44.9%) general physicians, 72 (20.5%) internal medicine practitioners, 41 (11.6%) pediatricians, 17 (4.8%) gastroenterologists, 11 (3.1%) infectious disease specialists, and 53 (15.1%) other specialists. Regarding the responding method, 31.5% responded using face-to-face interviews, and 68.5% used an electronic questionnaire.

4.2. Physicians' Knowledge Regarding *Blastocystis hominis*

A high percentage of physicians (77.8%) knew that *B. hominis* is a parasite, but less than a third (29.5%) knew that *B. hominis* infection does not correlate with age. Regarding clinical symptoms, 64.2%, 58.0%, and 13.9% knew that *B. hominis* might cause abdominal pain, diarrhea, and urticaria, respectively. Approximately two-thirds (64.5%) of the study physicians knew that *B. hominis* colonize the human gut, but only 43.8% believed that *B. hominis* might be an emerging pathogen. Less than half of the physicians (36.4%) knew there was no correlation between *B. hominis* infections and occupation. Most physicians (91.8%) agreed that the fecal-oral route is the main mode of *B. hominis* transmission, and

63.4% believed that *B. hominis* had been linked to IBS. In addition, 64.2% knew there was no need to treat asymptomatic individuals (Table 1).

4.3. Physicians' Practice Regarding *Blastocystis hominis*

About 68% had a practice of treating infected immunocompromised patients, and 44.9% treated symptomatic patients. Among them, 64.5% reported that they prescribed metronidazole for treatment. In addition, 71.6% and 79.3% believed that *B. hominis* should be treated in symptomatic celiac and ulcerative colitis patients, respectively (Table 2).

4.4. Correlation Between Work Experience and Knowledge and Practice of Physicians Toward *Blastocystis hominis*

Work experience had a significant effect on physicians' knowledge about *B. hominis* ($r = 0.093$; $P = 0.008$), but the association between work experiences and practices of physicians regarding *B. hominis* was not significant ($r = 0.066$; $P = 0.061$).

5. Discussion

The study aimed to evaluate the knowledge level of Ahvaz's physicians about *B. hominis*. Although it has been more than 100 years since *Blastocystis* was recognized, our results indicated still a percentage of physicians (16.2%) knew this protozoan as a fungus *Blastocystis hominis* was first classified as yeast, then as a protest, and as Stramenopiles (11). Due to insufficient knowledge about this parasite, the treatment of infected people is still controversial (11); however, knowing that *B. hominis* is a parasite can play an important role in adopting anti-parasitic treatment for patients with chronic symptoms. These findings are consistent with the study conducted by Berger et al. (2018) regarding Chagas disease, in which 97% of physicians knew it was a parasitic disease (15).

It was found that the majority of physicians were aware of the possibility of abdominal pain and diarrhea in some symptomatic patients, but they had little knowledge of urticaria in individuals infected with *B. hominis*. Some *B. hominis* STs, such as ST2 and ST3, may cause gastrointestinal and extraintestinal clinical manifestations such as chronic urticaria (16, 17). Lack of adequate knowledge of the skin manifestations caused by *B. hominis* may be due to the low reported urticaria cases in infected individuals (6, 18, 19). Another possible explanation is that *B. hominis* is less mentioned in educational programs and training courses in Iran.

This study found that around 92% of physicians knew the oral-fecal route as the main transmission route of *B.*

hominis, but their knowledge of transmission through animal contact was low. This parasite is mainly transmitted through the oral-fecal route, but contact with animal hosts can also play a role in its transmission (11, 20). In agreement with our results, in a study conducted by Efunshile et al., only 35% of healthcare professionals were aware that humans infected with *Toxoplasma gondii* could occur by consuming undercooked meat of infected animals (21). Adequate knowledge of parasites and their transmission routes, such as *B. hominis*, is important in preventing and controlling this enteric protozoan (22).

Although some evidence suggests that this protozoan is more common in the gut of healthy individuals, it is also recognized as a potential pathogen associated with IBS (20). IBS, a common gastrointestinal disorder with a worldwide distribution of 11.2%, has multiple etiologies. The pathogenesis of the disease is not fully understood, but it is believed that several factors, such as host-related factors, psychological state, and pathogens (bacterial, virus, parasites), are involved in increasing the risk of the development of IBS (23). Approximately 64% of physicians believe that *B. hominis* might be linked to IBS. It seems that being aware of a likely association between *B. hominis* and IBS can affect the treatment of the disease. A recent study conducted on Indonesian adolescents suggested *Blastocystis* ST1 as a pathogenic subtype of the IBS-D type (24).

In the present study, 64.2% of physicians believed that there is no need for treatment in the absence of clinical symptoms, but the noteworthy point is that only 30.7% of the physicians agreed to treat symptomatic patients. On the other hand, physicians' practice of treating symptomatic patients was also poor. Insufficient knowledge of the physicians about the pathogenicity of this parasite could be the reason for the poor practice of doctors in treating symptomatic patients.

In the present study, 64.5% of physicians recommended metronidazole as a first-line treatment option for *B. hominis*. Metronidazole is the most frequently recommended drug for *B. hominis* infection (11); however, resistance to metronidazole has been reported in various studies (11, 25, 26). For this reason, the necessity of metronidazole reassessment is recommended (26).

We found a significant correlation between years of experience and physicians' knowledge of *B. hominis*.

This study had limitations. First, due to the COVID-19 pandemic, we could not assess the knowledge and practice of all physicians about *Blastocystis* using a face-to-face questionnaire. The second limitation was that the grouping of physicians in terms of the specialty did not have a normal distribution.

Table 1. Knowledge of *Blastocystis hominis* Among Physicians (N = 352) Participating in This Study

Question (Group)	Yes, No. (%)	No, No. (%)
What is your opinion about <i>B. hominis</i>?		
It colonizes the gut	227 (64.5)	125 (35.5)
It is a non-pathogenic protist	44 (12.5)	308 (87.5)
All infected people are asymptomatic	32 (9.1)	320 (90.9)
It might be an emerging pathogen	154 (43.8)	198 (56.3)
Which occupation group is more likely to be infected with <i>B. hominis</i>?		
Rancher	127 (36.1)	225 (63.9)
Food seller	84 (23.9)	268 (76.1)
Farmer	50 (14.2)	302 (85.8)
No correlation between occupation	128 (36.4)	224 (63.6)
What are the common modes of <i>B. hominis</i> transmission?		
Fecal-oral	323 (91.8)	29 (8.2)
Blood transfusion	3 (0.9)	349 (99.1)
Congenital	5 (1.4)	347 (98.6)
Exposure to infected animals	63 (17.9)	289 (82.1)
Sexual	9 (2.6)	343 (97.4)
Airborne	20 (5.7)	332 (94.3)
Which disease may <i>B. hominis</i> be linked to?		
Celiac	61 (17.3)	291 (82.7)
IBS	223 (63.4)	129 (36.6)
Ulcerative colitis	68 (19.3)	284 (80.7)
Asthma	54 (15.3)	298 (84.7)
What is true about <i>B. hominis</i>?		
Asymptomatic individuals do not need to be treated	226 (64.2)	126 (35.8)
It may be a marker of gastrointestinal health	22 (6.3)	330 (93.8)
Symptomatic patients should be treated	108 (30.7)	244 (69.3)
In IBS patients, <i>B. hominis</i> may reduce gastrointestinal symptoms	37 (10.5)	315 (89.5)

5.1. Conclusions

This study showed that physicians had adequate knowledge about the parasitic nature of *B. hominis*, its most common route of transmission, as well as some of the common clinical symptoms caused by it, such as abdominal pain and diarrhea; however, their knowledge was poor about the role of host animals in its transmission, the need to treat symptomatic patients, the possibility of extraintestinal clinical symptoms such as urticaria, and resistance to metronidazole in some cases. Furthermore, the obtained results indicated that physicians' practices in treating symptomatic patients were also poor. This emphasizes the need to improve physicians' knowledge of the pathogenicity and treatment of *B. hominis*. Since *B. hominis* is a common protozoan with pathogenic potential,

we recommend training sessions for physicians to inform them of new findings about *B. hominis*.

Acknowledgments

We would like to thank all participants. The data collection was performed by M Fasihi Karami.

Footnotes

Authors' Contribution: Conceived and designed the survey, MB, AR; Collection data, MF; Analyzed the data, BCh and EB; Writing, MB, AR.

Conflict of Interests: The authors declare that they have no competing interests.

Table 2. Practice of Participating Physicians (N = 352) on *Blastocystis hominis* in This Study

Question (Group)	Yes, No. (%)	No, No. (%)
Which group should be treated if <i>B. hominis</i> was the only organism reported in stool examination?		
Immunocompromised patients	239 (67.9)	113 (32.1)
Symptomatic patients	158 (44.9)	194 (55.1)
Children	51 (14.5)	301 (85.5)
Pregnant women	33 (9.4)	319 (90.6)
If the patient needs treatment, which of the following medications do you prescribe?		
Metronidazole	227 (64.5)	125 (35.5)
Iodoquinol	48 (13.6)	304 (86.4)
Azithromycin	35 (9.9)	317 (90.1)
Paromomycin	61 (17.3)	291 (82.7)
Do you think <i>B. hominis</i> should be treated in celiac patients?		
Symptomatic patients should be treated	252 (71.6)	100 (28.4)
No need to treat symptomatic patients	93 (26.4)	259 (73.6)
Do you think <i>B. hominis</i> should be treated in ulcerative colitis patients?		
Symptomatic patients should be treated	279 (79.3)	73 (20.7)
No need to treat symptomatic patients	72 (20.5)	280 (79.5)

Ethical Approval: The protocol of the present study was reviewed and approved by the Ethics Committee of the Student Research Committee, Ahvaz Jundishapur University of Medical Sciences ([IR.AJUMS.REC.1398.836](https://doi.org/10.1016/B978-0-12-407706-5.00001-0)). The methods were carried out in accordance with relevant guidelines and regulations.

Funding/Support: This study was supported by the Student Research Committee, Ahvaz Jundishapur University of Medical Sciences (grant No. 98s80).

Informed Consent: Informed consent was taken from all of the participants.

References

- Clark CG, van der Giezen M, Alfellani MA, Stensvold CR. Recent developments in Blastocystis research. *Adv Parasitol.* 2013;**82**:1-32. [PubMed ID: 23548084]. <https://doi.org/10.1016/B978-0-12-407706-5.00001-0>.
- Jimenez PA, Jaimes JE, Ramirez JD. A summary of Blastocystis subtypes in North and South America. *Parasit Vectors.* 2019;**12**(1):376. [PubMed ID: 31358042]. [PubMed Central ID: PMC6664531]. <https://doi.org/10.1186/s13071-019-3641-2>.
- Andersen LO, Stensvold CR. Blastocystis in health and disease: Are we moving from a clinical to a public health perspective? *J Clin Microbiol.* 2016;**54**(3):524-8. [PubMed ID: 26677249]. [PubMed Central ID: PMC4767957]. <https://doi.org/10.1128/JCM.02520-15>.
- Yason JA, Liang YR, Png CW, Zhang Y, Tan KSW. Interactions between a pathogenic Blastocystis subtype and gut microbiota: in vitro and in vivo studies. *Microbiome.* 2019;**7**(1):30. [PubMed ID: 30853028]. [PubMed Central ID: PMC6410515]. <https://doi.org/10.1186/s40168-019-0644-3>.
- Dogruman-Al F, Kustimur S, Yoshikawa H, Tuncer C, Simsek Z, Tanyuksel M, et al. Blastocystis subtypes in irritable bowel syndrome and inflammatory bowel disease in Ankara, Turkey. *Mem Inst Oswaldo Cruz.* 2009;**104**(5):724-7. [PubMed ID: 19820833]. <https://doi.org/10.1590/s0074-02762009000500001>.
- Casero RD, Mongi F, Sanchez A, Ramirez JD. Blastocystis and urticaria: Examination of subtypes and morphotypes in an unusual clinical manifestation. *Acta Trop.* 2015;**148**:156-61. [PubMed ID: 25976414]. <https://doi.org/10.1016/j.actatropica.2015.05.004>.
- Shirvani G, Fasihi-Harandi M, Raiesi O, Bazargan N, Zahedi MJ, Sharifi I, et al. Prevalence and molecular subtyping of Blastocystis from patients with irritable bowel syndrome, inflammatory bowel disease and chronic urticaria in Iran. *Acta Parasitol.* 2020;**65**(1):90-6. [PubMed ID: 31602552]. <https://doi.org/10.2478/s11686-019-00131-y>.
- Rudzinska M, Kowalewska B, Szostakowska B, Grzybek M, Sikorska K, Swiatalska A. First report on the occurrence and subtypes of Blastocystis in Pigs in Poland using sequence-tagged-site PCR and barcode region sequencing. *Pathogens.* 2020;**9**(7). [PubMed ID: 32708299]. [PubMed Central ID: PMC7400023]. <https://doi.org/10.3390/pathogens9070595>.
- Wawrzyniak I, Poirier P, Viscogliosi E, Dionigia M, Texier C, Delbac F, et al. Blastocystis, an unrecognized parasite: an overview of pathogenesis and diagnosis. *Ther Adv Infect Dis.* 2013;**1**(5):167-78. [PubMed ID: 25165551]. [PubMed Central ID: PMC4040727]. <https://doi.org/10.1177/2049936113504754>.
- Stensvold CR, Clark CG. Current status of Blastocystis: A personal view. *Parasitol Int.* 2016;**65**(6 Pt B):763-71. [PubMed ID: 27247124]. <https://doi.org/10.1016/j.parint.2016.05.015>.
- Roberts T, Stark D, Harkness J, Ellis J. Update on the pathogenic potential and treatment options for Blastocystis sp. *Gut Pathog.* 2014;**6**:17. [PubMed ID: 24883113]. [PubMed Central ID: PMC4039988]. <https://doi.org/10.1186/1757-4749-6-17>.
- Badparva E, Ezatpour B, Mahmoudvand H, Behzadifar M, Behzadifar M, Kheirandish F. Prevalence and Genotype Analysis of Blastocystis hominis in Iran: A Systematic Review and Meta-Analysis. *Arch Clin Infect Dis.* 2016;**12**(1). e36648. <https://doi.org/10.5812/archcid.36648>.

13. Khademvatan S, Masjedizadeh R, Yousefi-Razin E, Mahbodfar H, Rahim F, Yousefi E, et al. PCR-based molecular characterization of *Blastocystis hominis* subtypes in southwest of Iran. *J Infect Public Health*. 2018;**11**(1):43-7. [PubMed ID: 28404232]. <https://doi.org/10.1016/j.jiph.2017.03.009>.
14. Salehi R, Haghighi A, Stensvold CR, Kheirandish F, Azargashb E, Raeghi S, et al. Prevalence and subtype identification of *Blastocystis* isolated from humans in Ahvaz, Southwestern Iran. *Gastroenterol Hepatol Bed Bench*. 2017;**10**(3):235-41. [PubMed ID: 29118941]. [PubMed Central ID: PMC5660275].
15. Berger BA, Bartlett AH, Jimenez-Hernandez R, Trinidad Vazquez E, Galindo-Sevilla N. Physician knowledge, attitudes, and practices related to chagas disease in Tabasco, Mexico. *Am J Trop Med Hyg*. 2018;**98**(6):1743-7. [PubMed ID: 29692299]. [PubMed Central ID: PMC6086191]. <https://doi.org/10.4269/ajtmh.17-0495>.
16. Vogelberg C, Stensvold CR, Monecke S, Ditzen A, Stopsack K, Heinrich-Grafe U, et al. *Blastocystis* sp. subtype 2 detection during recurrence of gastrointestinal and urticarial symptoms. *Parasitol Int*. 2010;**59**(3):469-71. [PubMed ID: 20363362]. <https://doi.org/10.1016/j.parint.2010.03.009>.
17. Aykur M, Camyar A, Turk BG, Sin AZ, Dagci H. Evaluation of association with subtypes and alleles of *Blastocystis* with chronic spontaneous urticaria. *Acta Trop*. 2022;**231**:106455. [PubMed ID: 35413246]. <https://doi.org/10.1016/j.actatropica.2022.106455>.
18. Pasqui AL, Savini E, Saletti M, Guzzo C, Puccetti L, Auteri A. Chronic urticaria and *Blastocystis hominis* infection: a case report. *Eur Rev Med Pharmacol Sci*. 2004;**8**(3):117-20. [PubMed ID: 15368795].
19. Melo GB, Malta FM, Maruta CW, Criado PR, Castilho VLP, Goncalves E, et al. Characterization of subtypes of *Blastocystis* sp. isolated from patients with urticaria, Sao Paulo, Brazil. *Parasite Epidemiol Control*. 2019;**7**. e00124. [PubMed ID: 31872093]. [PubMed Central ID: PMC6911935]. <https://doi.org/10.1016/j.parepi.2019.e00124>.
20. Lhotska Z, Jirku M, Hlozkova O, Brozova K, Jirsava D, Stensvold CR, et al. A study on the prevalence and subtype diversity of the intestinal Protist *Blastocystis* sp. in a gut-healthy human population in the Czech Republic. *Front Cell Infect Microbiol*. 2020;**10**:544335. [PubMed ID: 33123491]. [PubMed Central ID: PMC7573152]. <https://doi.org/10.3389/fcimb.2020.544335>.
21. Efunshile MA, Onwakpu KO, Robertson LJ, Jokelainen P. Opinions and knowledge on globally important foodborne parasites among healthcare professionals at a tertiary teaching hospital in Nigeria. *Food Waterborne Parasitol*. 2020;**18**. e00075. [PubMed ID: 32154397]. [PubMed Central ID: PMC7058820]. <https://doi.org/10.1016/j.fawpar.2020.e00075>.
22. Khan A, Naz K, Ahmed H, Simsek S, Afzal MS, Haider W, et al. Knowledge, attitudes and practices related to cystic echinococcosis endemicity in Pakistan. *Infect Dis Poverty*. 2018;**7**(1):4. [PubMed ID: 29353554]. [PubMed Central ID: PMC5776779]. <https://doi.org/10.1186/s40249-017-0383-2>.
23. Cifre S, Gozalbo M, Ortiz V, Soriano JM, Merino JF, Trelis M. *Blastocystis* subtypes and their association with Irritable Bowel Syndrome. *Med Hypotheses*. 2018;**116**:4-9. [PubMed ID: 29857906]. <https://doi.org/10.1016/j.mehy.2018.04.006>.
24. Kesuma Y, Firmansyah A, Bardosono S, Sari IP, Kurniawan A. *Blastocystis* ST-1 is associated with Irritable Bowel Syndrome-diarrhoea (IBS-D) in Indonesian adolescences. *Parasite Epidemiol Control*. 2019;**6**. e00112. [PubMed ID: 31528737]. [PubMed Central ID: PMC6742775]. <https://doi.org/10.1016/j.parepi.2019.e00112>.
25. Mirza H, Teo JD, Upcroft J, Tan KS. A rapid, high-throughput viability assay for *Blastocystis* spp. reveals metronidazole resistance and extensive subtype-dependent variations in drug susceptibilities. *Antimicrob Agents Chemother*. 2011;**55**(2):637-48. [PubMed ID: 21098237]. [PubMed Central ID: PMC3028762]. <https://doi.org/10.1128/AAC.00900-10>.
26. Rajamanikam A, Hooi HS, Kudva M, Samudi C, Kumar S. Resistance towards metronidazole in *Blastocystis* sp.: A pathogenic consequence. *PLoS One*. 2019;**14**(2). e0212542. [PubMed ID: 30794628]. [PubMed Central ID: PMC6386359]. <https://doi.org/10.1371/journal.pone.0212542>.