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# *Blastocystis* subtypes in patients with diabetes mellitus from the Midwest region of Brazil

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## ABSTRACT

*Blastocystis* sp. is an enteric protist commonly found in human fecal samples. In Brazil, few studies have been developed, but none of them has explored the presence of *Blastocystis* in patients with diabetes mellitus. We evaluated the occurrence and molecular identification of *Blastocystis* sp. among patients with diabetes mellitus in the Midwest region, Goias State, Brazil. Genomic DNA was obtained from 175 fecal samples (99 from the diabetic group and 76 from the control group). PCR was performed using pan-*Blastocystis* primers from the SSU-rDNA gene. Microscopic examination revealed positivity of 12.1% and 7.9% for *Blastocystis* in diabetics and in controls, respectively. Amplification of *Blastocystis* DNA was observed in 34.4% (34 of 99) and 30.3% (23 of 76) from the diabetic and control groups, respectively. Phylogenetic analyses and BLAST searches revealed six subtypes among *Blastocystis* isolates in the diabetic group, represented by ST1 (38.2%), ST2 (11.8%), ST3 (35.3%), ST6 (2.9%), ST7 (2.9%) and ST8 (8.8%). In the control group, ST1 (21.8%), ST2 (21.8%), ST3 (43.5%), ST6 (4.4%) and ST8 (8.7%) were identified. This study is the first report regarding the occurrence and subtypes distribution of *Blastocystis* in patients with diabetes mellitus in Brazil. The results reinforce the potential risk of *Blastocystis* infection in patients with diabetes, in addition, it contributes to the understanding of the genetic diversity of this enigmatic organism.

**KEYWORDS:** *Blastocystis* sp. Diabetes mellitus. Subtype. Allele. Brazil.

## INTRODUCTION

Diabetes is a metabolic disease that has become more frequent over the years, and has a worldwide distribution[1]. Type 2 diabetes (T2D) is responsible for approximately 90% of the cases of diabetes mellitus[2]. This disease has been considered a serious health problem in Brazil and worldwide, both in terms of the number of affected people, as well as disabilities and premature deaths[2-4].

Intestinal parasites continue to be considered an important public health problem worldwide, responsible for high morbidity and mortality, especially in developing countries[1,5]. *Blastocystis* sp. has been highly reported in routine parasitological examinations, being one of the most prevalent enteric protists in human fecal samples[6]. On the other hand, its ability to cause disease is debated, and it is a subject of ongoing research[7]. Molecular studies have demonstrated a variable genetic diversity with description of 17 distinct subtypes (STs) to date, 10 of which have been found in humans (ST1-9 and ST12)[7,8]. In addition, it has been shown that certain subtypes can be considered more pathogenic to humans than others[6,7].

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Intestinal parasites and diabetes have been constantly evaluated, mainly due to the possibility of immune dysfunction in diabetes allowing the development of more severe parasitic infections[9]. In this context, certain parasitic infections can occur more frequently in patients with diabetes mellitus than in non-diabetics[1,5,10]. On the other hand, the occurrence of *Blastocystis* sp. is little known, especially in patients with diabetes[1,5]. Studies carried out in the Midwest region of Brazil indicate positivity ranging from 0.5 to 40.9% for *Blastocystis* sp., but none of them analyzed patients with diabetes[11-14]. Here, we present data on the occurrence and on the molecular identification of *Blastocystis* sp. in fecal samples of patients with diabetes mellitus living in Jatai, Goias State, Brazil.

## MATERIALS AND METHODS

### Ethical Statement

The present study was approved by the Ethics and Research Committee of Universidade Federal de Goias (protocol Nº 929187/2015). After written informed consent was obtained, one fecal sample from each participant was collected.

### Study site

This study was carried out from January 2015 to December 2016 in the Jatai municipality, Goias State, which is located in the Midwest region of Brazil (17º52'33"S, 51º43'17"W), 535 km from Brasilia (the capital of Brazil). Jatai has a total population of 100,882 inhabitants[15].

### Sampling

This study included unpreserved fecal samples from individuals of both genders, with ages ranging from 18 to 89 years old. The individuals were divided into two groups:

- The diabetic group (n = 99) was composed of patients with type 2 diabetes mellitus (T2D) attending the Program of Education and Control of Diabetes in the Basic Health Unit of Jatai municipality. Inclusion criteria of the diabetic group were the diagnosis of DM2; blood tests associated to the diabetes control performed in the last two years; glycated haemoglobin (HbA1c) > 6.5%.
- Inclusion criteria of the diabetic group were the diagnosis of DM2, and > 30 years old; blood tests associated to the diabetes control performed in the last two years.
- The control group ($n$ = 76) included T2D patients' companions or other patients in treatment at the Basic Health Unit, who have declared not to have T2D; had the glycated haemoglobin (HbA1c) evaluated with results < 6.5% and have not used anthelmintic/antiprotozoal drugs in the last year.

Fecal samples were immediately transported to the Laboratorio de Parasitologia, Universidade Federal de Jataí in Goias State, and processed within 12 h of collection by Lutz and Baermann modified methods for microscopic examination[16]. A second aliquot was immediately frozen at -20 °C for extracting genomic DNA. This subsample set was shipped to Laboratorio de Investigacao Medica (LIM/06), Hospital das Clinicas of the Faculdade de Medicina of the Universidade de Sao Paulo, for molecular analysis.

### DNA extraction and PCR amplification

Genomic DNA was extracted directly from fecal samples using the QIAamp Stool Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's recommendations. DNA was eluted in a final volume of 100 µL and stored at -20ºC in the freezer until the PCR (Polymerase Chain Reaction) analysis. DNA isolates were analysed for the presence of *Blastocystis* sp. through PCR performed with pan-*Blastocystis* primers RD5 (5'-ATCTGGTTGATCCTGCCAGT-3') and BhRDr (5'-GAGCTTTTAACTGCAACAA CG-3') described by Scicluna *et al.*[17], that amplify a DNA fragment of approximately 600 bp from the *SSU* rDNA gene.

Four microliters of the DNA solution were added into the standard PCR mixture with a total reaction volume of 20 µL. GoTaq® DNA Polymerase (5 U/µL, Promega Corporation, Madison, WI, USA) was used in all the PCR reactions. Amplification cycles were composed of an initial denaturation step of 94 °C for 2 min, followed by 30 cycles of 94 ºC for 1 min (denaturation), 61.8 ºC for 1 min (annealing) and 72 ºC for 1 min (extension), and a final extension of 72 ºC for 2 min. In all PCR amplifications, a positive control (PCR mixture with *Blastocystis* sp. culture isolate) and a negative control (PCR mixture with non-template water) were included. Amplification products were analysed on 2% agarose gel electrophoresis stained with Sybr Safe (Invitrogen™, Thermo Fisher Scientific Corporation, Waltham, USA) and visualized under UV light.

### Subtyping and phylogenetic analyses of *Blastocystis* sp.

PCR products were purified using the Exo-SAP-IT™ PCR clean-up (USB Corporation, Cleveland, Ohio, USA)

and sequenced by the two-directional sequencing for increasing the sequencing accuracy using the BigDye Terminator v3.1 cycle sequencing kit and the same primers used in the PCR. Sequencing data were generated on an ABI 3500 automated DNA sequencer (Applied Biosystems, Thermo Fisher Scientific, Waltham, USA).

The quality of sequences was evaluated using the Phred-Phrap software and the consensus sequence of each sample was assembled using the CAP3 software available at the Electropherogram quality analysis webpage[18]. Sequences were aligned and edited when necessary using CLUSTAL W and BioEdit v.7.0.8 software programs (Ibis Bioscience, Carlsbad, CA, USA).

For subtypes identification the consensus sequence of each sample was analyzed using the Basic Local Alignment Search Tool (BLASTn)[19]. Sequences were also submitted to phylogenetic analysis together with published reference sequences from the GenBank database to confirm the subtype classification. Subtype identification and allele discrimination based on 18S rRNA gene of *Blastocystis* were performed using the *Blastocystis* database[20].

Phylogenetic analysis was performed with the Mega software (Version 10) through the Molecular Evolutionary Genetics Analysis package, using the Maximum Likelihood method and Tamura 3-parameter model[21]. Genetic distances were calculated using Kimura's two-parameter model. Bootstrap analysis with 1,000 replicates was performed to test the reliability of the tree with values ≥ 70 indicated on the branches.

### Data analysis

The agreement between microscopy and molecular analyses was measured using the Cohen's kappa coefficient (κ) (GraphPad Prism®, Melbourne, Australia). The data were analyzed using the Fisher's exact test to compare the frequency data generated by the study groups. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were used. A $p$ value < 0.05 was considered statistically significant.

## RESULTS

Fecal samples were collected from a total of 175 individuals. The diabetic group was composed of 38 (38.4%) males and 61 (61.6%) females, ranging from 32 to 87 years (62.8 ± 10.5 years), with a mean HbA1c of 8.9% (ranging from 6.8 to 16.8%) The control group was composed of 33 (43.4%) males and 43 (56.6%) females, ranging from 21 to 89 years (56.6 ± 18.5 years). There was no statistically significant difference in the comparison of variables between the two groups.

Overall, the microscopic examination revealed the presence of parasites in 31.3% (31/99) of the diabetic patients who harbored at least one intestinal parasite/commensal species. The most predominant species were non-pathogenic amebae (42.42%, 42/99); followed by *Blastocystis* sp. (12.1%, 12/99), *Entamoeba hystolytica* complex (2.02%, 2/99), *Giardia intestinalis* (1.01%, 1/99), and by helminths *Strongyloides stercoralis* and *Hymenolepsis nana* (1.01%, 1/99). In the control group, the microscopic examination showed that 25% (19/76) of fecal samples had at least one intestinal parasite/commensal species. Among identified species, there were non-pathogenic amebae (36.84%, 28/76); followed by *Blastocystis* sp. (7.89%, 6/76), *Entamoeba hystolytica* complex (5.26%, 4/76), and by helminths *S. stercoralis* and *Trichuris trichiura* (1.32%, 1/76).

PCR products of the expected size (around 600 bp) were observed in 44.4% (44/99) and in 34.2% (26/76) of the DNA samples from the diabetic and control groups, respectively, $p > 0.05$. Among the 70 PCR-positive samples, 57 (34 and 23 from the diabetic and control groups, respectively) were confirmed to be positive to *Blastocystis* by the sequencing of the amplification product. A total of 13 samples were excluded due to poor sequence quality (n = 7) and no similarity with *Blastocystis* in GenBank (n = 6). Additional analyses are being conducted to accurately determine their taxonomic identity.

Considering the results of the microscopic and molecular analyses, 36.0% (63/175) of the samples were positive for *Blastocystis*. Comparing the number of *Blastocystis*-positive samples identified by microscopy and molecular analyses, a low concordance was observed [κ = 0.194 (0.060–0.328)], $p < 0.001$. Six samples (three in each group) were *Blastocystis* sp.-positive by microscopy, nonetheless, they were negative by molecular analyses. Table 1 shows the distribution of diabetic and control participants according to age, gender and *Blastocystis*-positive results.

The 57 successfully subtyped sequences showed a high similarity (98.78 to 100%) in comparison with *Blastocystis* sequences reported in GenBank (Supplementary Table S1). The nucleotide sequences obtained in this study have been deposited in the GenBank database under the accession numbers: MN585810-MN585817, MN585819, MN585821-MN585822, MN585824, MN585826-MN585832, MN585834-MN585837, MN585839, MN585841-MN585847, MN585849-MN585850, MN585852, MN585854-MN585864, MN585866-MN585867 and MN585870-MN585879.

The nucleotide sequence analysis revealed the presence of six subtypes among the diabetic isolates: ST1 (13/34;

**Table 1 -** Association between the positivity to *Blastocystis* sp. and characteristics of the diabetic and the control group.

| | Diabetic group | Control group | *P* value | OR (95% CI ) |
|---|---|---|---|---|
| **Characteristics** | | | | |
| Gender* | | | | |
| ♂ | 15 (40.5%) | 14 (46.1%) | 0.8060 | 0.8866 (0.3382-2.323) |
| ♀ | 22 (59.5%) | 12 (53.9%) | 0.3938 | 1.452 (0.6226 -3.474) |
| Age* | | | | |
| < 65 years | 14 (37.8%) | 16 (61.5%) | 0.3328 | 0.6539 (0.2745-1.544) |
| ≥ 65 years | 23 (62.2%) | 10 (38.5%) | 0.1278 | 2.154 (0.8054 -5.955) |
| **Results** | | | | |
| Microscopy | 12 (12.1%) | 6 (7.9%) | 0.3779 | 1.605 (0.5774 -4.846) |
| PCR | 34 (34.4%) | 23 (30.3%) | 0.5745 | 1.204 (0.6332 -2.309) |

*considering the results of microscopy and the molecular analyses

38.2%), ST2 (4/34; 11.8%), ST3 (12 /34; 35.3%), ST6 (1/34; 2.9%), ST7 (1/34; 2.9%) and ST8 (3/34; 8.8%). Subsequently, we identified the alleles present in each subtype from isolates of the diabetic group: ST1 (allele 4), ST2 (alleles 9 and 12), ST3 (alleles 34, 36 and 37), ST6 (allele 122), ST7 (allele 99) and ST8 (allele 21) (Table 2, Supplementary Table S1). In the control group, five subtypes were identified: ST1 (5/23; 21.8%), ST2 (5/23; 21.8%), ST3 (10/23; 43.5%), ST6 (1/23; 4.4%) and ST8 (2/23; 8.7%). The following were identified in the ST1 (allele 4), ST2 (alleles 11 and 15), ST3 (alleles 34 and 36), ST6 (allele 134) and ST8 (allele 21) (Table 2, Supplementary Table S1). The higher occurrence of ST1 in diabetic isolates in relation to the control group (72.2 vs 27.8%; p = 0.01030) can be observed. There was no significant difference between the other subtypes.

Based on the barcode sequence the similarity of the sequences within each subtype was calculated and showed a range from 99.987 to 100%. Considering the inter-subtypes ST1 and ST2 (99.974) shows highest similarity, while ST2 and ST7 (99.849) shows lowest similarity. To establish a probable correlation between diabetic and intra-subtype variation, genetic similarity between sequences of diabetic and controls isolates among each subtype was calculated, and it showed the lowest similarity in ST2 (99.994%) versus the highest similarity in ST3 isolates (100%).

A Phylogenetic tree was constructed, and it revealed that all six subtypes were clearly separated (Figure 1). Moreover, the tree indicated that sequences obtained from the *Blastocystis* isolates from the diabetic group were not separated from those from the control group. This suggests that there was no association between the presence of diabetes and *Blastocystis* sp.

## DISCUSSION

*Blastocystis* sp. has been one of the most prevalent anaerobic protists found in human fecal specimens[22]. However, it still remains surrounded by many uncertainties, especially regarding its pathogenic potential[7]. Patients with diabetes mellitus can present intestinal parasites infections with more serious complications[5]. In addition, the literature

**Table 2 -** Distribution of *Blastocystis* sp. subtypes and alleles in the diabetic and the control group.

| Subtypes | Diabetic group | | | Control group | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Alleles | *n* | (%) | Alleles | *n* | (%) | Alleles | *n* | (%) |
| ST1 | 4 | 13/13 | 100 | 4 | 5/5 | 100 | 4 | 18/18 | 100 |
| ST2 | 9 | 1/4 | 25 | - | - | - | 9 | 1/9 | 11.1 |
| | - | - | - | 11 | 3/5 | 60 | 11 | 3/9 | 33.3 |
| | 12 | 3/4 | 75 | - | - | - | 12 | 3/9 | 33.3 |
| | - | - | - | 15 | 2/5 | 40 | 15 | 2/9 | 22.2 |
| ST3 | 34 | 6/12 | 50 | 34 | 8/10 | 80 | 34 | 14/22 | 63.6 |
| | 36 | 2/12 | 16.7 | 36 | 2/10 | 20 | 36 | 4/22 | 18.2 |
| | 37 | 4/12 | 33.3 | - | - | - | 37 | 4/22 | 18.2 |
| ST6 | 122 | 1/1 | 100 | - | - | - | 122 | 1/2 | 50 |
| | - | - | - | 134 | 1/1 | 100 | 134 | 1/2 | 50 |
| ST7 | 99 | 1/1 | 100 | - | - | - | 99 | 1/1 | 100 |
| ST8 | 21 | 3/3 | 100 | 21 | 2/2 | 100 | 21 | 5/5 | 100 |

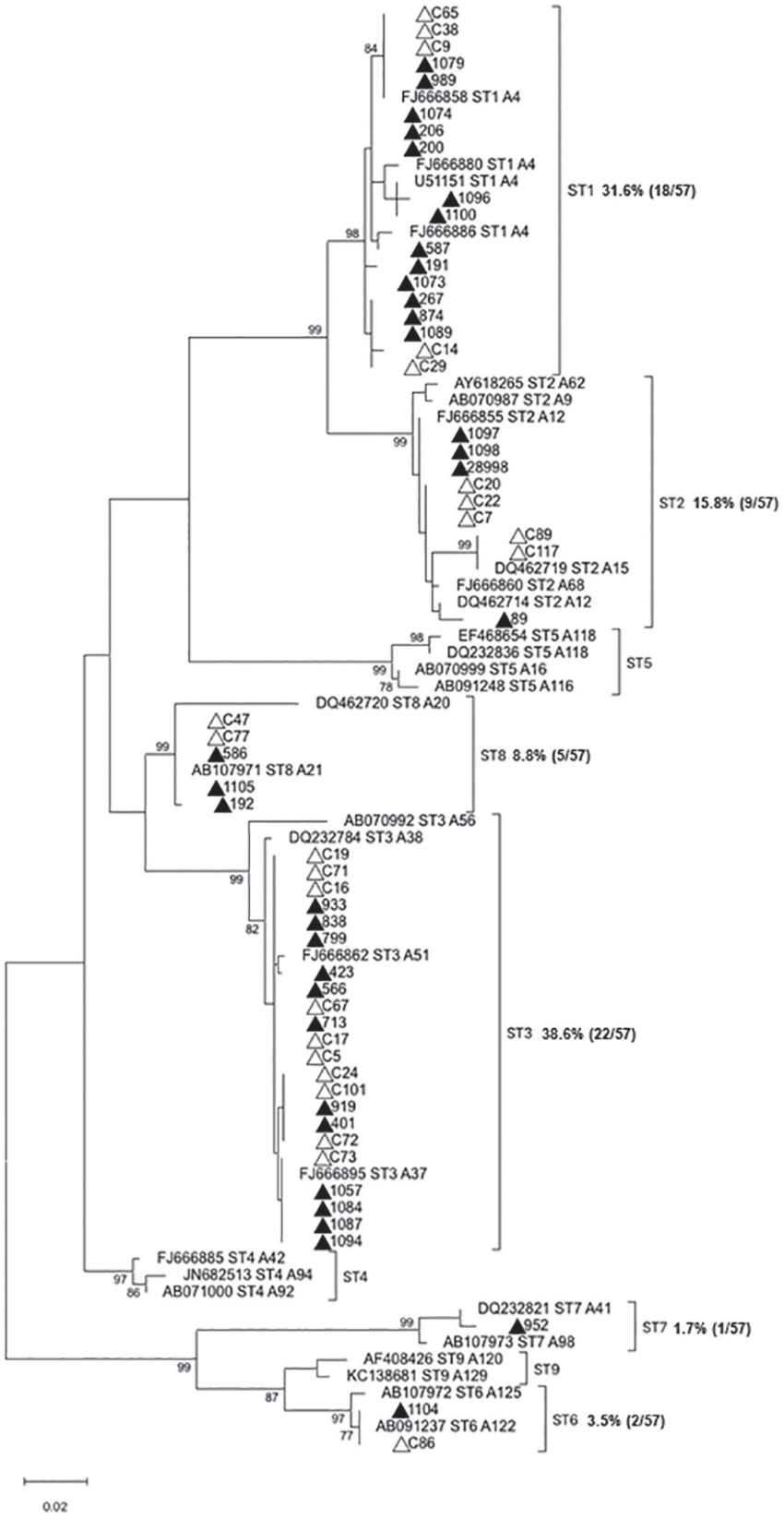


**Figure 1** - Phylogenetic analysis of *Blastocystis* SSU rDNA sequences (around 600bp) generated in the diabetic group (identified by filled black triangles) and the control group (identified by empty black triangles). Reference sequences from GenBank (identified by accession numbers and subtypes). The phylogenetic tree was constructed using the Maximum Likelihood method. Bootstrap values < 70% are not shown. The subtype prevalence in the 57 samples is shown on the right as a percentage.

has been emphasized the high frequency of protozoan and helminths in patients with type 2 diabetes in relation to type 1 diabetes[10]. It is worth noting that, to our knowledge, there are no studies conducted in Brazil that explored the occurrence of *Blastocystis* sp. in patients with diabetes mellitus.

Parasitological techniques by microscopy are used as the main diagnostic tool for *Blastocystis* in the majority of laboratories. In the present study, *Blastocystis* positivity by microscopic examination of 12.1% and 7.9% were observed in the diabetic and the control group, respectively. This positivity was lower in previous studies[12,23] and higher in others[11,13,14] carried out in the Midwest region of Brazil. In addition, Bafghi *et al*.[5] demonstrated inferior results (2.4%) in diabetic patients' from Iran. In our study, we used parasitological techniques employed in the routine of clinical laboratories that may have contributed to the low positivity. However, some authors have reported the importance of using stained smears and culture methods for the diagnosis of *Blastocystis*[6,22], mainly to improve the visualization and distinction of *Blastocystis* forms[6].

Poor quality sequences were not considered in the genetic analysis. These sequences may occur due to incomplete replication of the DNA strand during the cycle as a result of a PCR product combining sequences from the two sources (different organisms)[24]; or due to the extensive sequence similarity in some regions of the SSU rDNA[25]. Thus, the positivity of *Blastocystis* should always be confirmed by sequencing.

Molecular analysis (34.4% and 30.3% in the diabetic and the control groups, respectively) showed a higher positivity in relation to the microscopic examination. One possible explanation is that the non-use of high sensitivity parasitological techniques to detect this protist[22]. On the other hand, it is important to highlight the occurrence of some positive parasitological samples that were PCR-negative. Isolates that were not amplified by pan-*Blastocystis* primers (barcode region) or the presence of PCR inhibitors in fecal samples can be considered to explain the non-amplification of *Blastocystis* DNA[26]. In addition, in *Blastocystis* research, molecular tools can have a crucial impact on the understanding of its epidemiology, genetic diversity and transmission[27]. Thus, our findings reinforce the usefulness of PCR, followed by sequencing, as a sensitive diagnostic method for the detection of this organism.

In the last years, a variety of studies have been developed to identify *Blastocystis* sp. subtypes that were circulating in the human population[7,8]. Through the molecular identification of the barcode region, six subtypes were identified: ST1, ST2, ST3, ST6, ST7 and ST8. Globally, subtypes ST1-ST4 have been identified as the most common in humans[7,8]. It is interesting to note that there was a predominance of ST1, followed by ST3 and ST8 in diabetics, whileST3 was observed followed by ST1 and ST2 in the control group. In the Midwest region, only one study[23] evaluated the presence of *Blastocystis* subtypes reinforcing the greater occurrence of ST1, followed by ST2 and ST3. In other regions of Brazil, the situation is similar to the results of the control group with the highest occurrence of ST3, followed by ST1 and ST2[28-31].

The identification of ST6-ST8 subtypes can characterize a potential area for zoonotic transmission. Subtypes 6 and 7 are usually found in birds, while ST8 is commonly found in non-human primates and it has also been reported in marsupials[8]. These subtypes are found sporadically in humans, with lower frequencies[7,8]. ST6 and ST8 were identified in other Brazilian isolates, recently reported by Melo *et al*.[30] and Seguí *et al*.[28], who analyzed clinical stool samples in Sao Paulo State and fecal samples of children in Parana State, respectively. Curiously, ST7 was detected only in the Brazilian studies carried out in the Southeast region[31,32]. In the present study, we observed relatively high occurrences of these subtypes, mainly ST8 (8.8% of diabetic and 8.7% of control isolates). Interestingly, in our results, there were no mixed infections among our isolates.

*Blastocystis* 18S alleles retrieved for each ST showed particular alleles in patients with diabetes mellitus, such as alleles 9 and 12 (ST2), allele 37 (ST3) and allele 122 (ST6), whereas in the control group isolates, alleles 11 and 15 (ST2) and allele 134 (ST6) were identified. The genetic diversity of alleles in this study was low when compared to studies already reported in Brazil[28,31,32]. Nonetheless, it should be noted that the allele 99 (ST7) was identified, and it had not yet been described in Brazil until now. Lastly, the presence of particular alleles associated with clinical implications needs to be further studied. In addition, different subtypes of *Blastocystis* appear to modulate the intestinal microbiota differently[33], and nutritional status of the individual may be an important risk factor[34].

Globally, the prevalence of *Blastocystis* infections in the two groups was similar in the present study, corroborating the results from a study carried out in Thailand[35], that evaluated the presence of *Blastocystis* in diabetics and non-diabetics. Nevertheless, other studies indicated that type 2 diabetes in humans can be associated with compositional changes in the intestinal microbiota[36], favouring the occurrence of *Blastocystis* in this group of patients.

The present study has some limitations that may have prevented us from reaching more robust conclusions, such as the accuracy of the parasitological diagnosis, as well as the lack of clinical and epidemiological data (location, previous contact with animals, presence or absence of

symptoms etc). Sociodemographic, hygienic and clinical aspects would have been very important, especially to understand the possible association between *Blastocystis* sp. Infections and patients with diabetes mellitus.

## CONCLUSIONS

In conclusion, the present study is the first report regarding *Blastocystis* sp. occurrence and subtype distribution. in patients with diabetes mellitus in Brazil. This protist was more identified in the T2D group than in the control group. Although we observed no association between *Blastocystis* infection and diabetes, the potential risk of *Blastocystis* infection should not be excluded. All things considered, our results update the distribution of the subtypes and alleles of *Blastocystis* sp. in Brazil.

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## AUTHORS' CONTRIBUTIONS

GBM and FMP conceived and designed the study; MCM, EAS, LVS, JEO and RMR collected the samples and carried out the clinical assessment; GBM performed the experiments; GBM, MGG and FMP carried out the analysis and interpretation of these data; GBM, FMP and RCBG drafted the manuscript. All authors read and approved the final manuscript.

## CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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**Supplementary Table S1** - Subtyping of Blastocystis isolates from diabetics and control groups.

| | Isolates | Genbank identification | Identity (%)* | Subtypes/Alleles | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | ST1 | ST2 | ST3 | ST6 | ST7 | ST8 |
| Diabetic group | 191 | MN585811.1 | 99.01[1] | 4 | - | - | - | - | - |
| | 200 | MN585813.1 | 99.17[1] | 4 | - | - | - | - | - |
| | 206 | MN585814.1 | 99.18[1] | 4 | - | - | - | - | - |
| | 267 | MN585815.1 | 99.30[1] | 4 | - | - | - | - | - |
| | 587 | MN585822.1 | 99.01[1] | 4 | - | - | - | - | - |
| | 874 | MN585828.1 | 99.31[1] | 4 | - | - | - | - | - |
| | 989 | MN585832.1 | 99.48[1] | 4 | - | - | - | - | - |
| | 1073 | MN585835.1 | 99.31[1] | 4 | - | - | - | - | - |
| | 1074 | MN585836.1 | 98.78[2] | 4 | - | - | - | - | - |
| | 1079 | MN585837.1 | 99.50[1] | 4 | - | - | - | - | - |
| | 1089 | MN585842.1 | 99.31[1] | 4 | - | - | - | - | - |
| | 1096 | MN585844.1 | 99.66[1] | 4 | - | - | - | - | - |
| | 1100 | MN585847.1 | 99.66[1] | 4 | - | - | - | - | - |
| | 89 | MN585810.1 | 98.89[3] | - | 12 | - | - | - | - |
| | 1097 | MN585845.1 | 99.30[3] | - | 12 | - | - | - | - |
| | 1098 | MN585846.1 | 99.34[3] | - | 12 | - | - | - | - |
| | 28998 | MN585852.1 | 99.30[3] | - | 9 | - | - | - | - |
| | 401 | MN585816.1 | 99.83[4] | - | - | 36 | - | - | - |
| | 423 | MN585817.1 | 99.83[4] | - | - | 34 | - | - | - |
| | 566 | MN585819.1 | 99.83[4] | - | - | 34 | - | - | - |
| | 713 | MN585824.1 | 99.67[4] | - | - | 34 | - | - | - |
| | 799 | MN585826.1 | 100[5] | - | - | 34 | - | - | - |
| | 838 | MN585827.1 | 99.83[4] | - | - | 34 | - | - | - |
| | 919 | MN585829.1 | 100[4] | - | - | 36 | - | - | - |
| | 933 | MN585830.1 | 100[4] | - | - | 34 | | - | - |
| | 1057 | MN585834.1 | 99.83[6] | - | - | 37 | - | - | - |
| | 1084 | MN585839.1 | 99.83[5,6] | - | - | 37 | - | - | - |
| | 1087 | MN585841.1 | 99.83[4,5,6] | - | - | 37 | - | - | - |
| | 1094 | MN585843.1 | 99.83[6] | - | - | 37 | - | - | - |
| | 1104 | MN585849.1 | 99.66[7] | - | - | - | 122 | - | - |
| | 952 | MN585831.1 | 100[8] | - | - | - | - | 99 | - |
| | 192 | MN585812.1 | 99.64[9] | - | - | - | - | - | 21 |
| | 586 | MN585821.1 | 99.82[9] | - | - | - | - | - | 21 |
| | 1105 | MN585850.1 | 99.82[9] | - | - | - | - | - | 21 |
| Control group | C9 | MN585856.1 | 99.50[1] | 4 | - | - | - | - | - |
| | C14 | MN585857.1 | 99.01[1] | 4 | - | - | - | - | - |
| | C29 | MN585864.1 | 99.34[1] | 4 | - | - | - | - | - |
| | C65 | MN585870.1 | 99.50[1] | 4 | - | - | - | - | - |
| | C38 | MN585866.1 | 99.50[1] | 4 | - | - | - | - | - |
| | C7 | MN585855.1 | 99.67[3] | - | 11 | - | - | - | - |
| | C20 | MN585861.1 | 99.66[3] | - | 11 | - | - | - | - |
| | C22 | MN585862.1 | 99.67[3] | - | 11 | - | - | - | - |
| | C89 | MN585877.1 | 99.003 | - | 15 | - | - | - | - |
| | C117 | MN585879.1 | 99.01[3] | - | 15 | - | - | - | - |
| | C5 | MN585854.1 | 99.83[4,5,6] | - | - | 34 | - | - | - |
| | C16 | MN585858.1 | 100[4] | - | - | 34 | - | - | - |
| | C17 | MN585859.1 | 99.67[4] | - | - | 34 | - | - | - |
| | C19 | MN585860.1 | 100[4] | - | - | 34 | - | - | - |
| | C24 | MN585863.1 | 100[5,6] | - | - | 36 | - | - | - |
| | C67 | MN585871.1 | 100[4] | - | - | 34 | - | - | - |
| | C71 | MN585872.1 | 100[4] | - | - | 34 | - | - | - |
| | C72 | MN585873.1 | 99.83[4,5,6] | - | - | 34 | - | - | - |
| | C73 | MN585874.1 | 99.83[4,5,6] | - | - | 34 | - | - | - |
| | C101 | MN585878.1 | 99.67[4] | - | - | 36 | - | - | - |
| | C86 | MN585876.1 | 99.49[7] | - | - | - | 134 | - | - |
| | C47 | MN585867.1 | 99.82[9] | - | - | - | - | - | 21 |
| | C77 | MN585875.1 | 99.82[9] | - | - | - | - | - | 21 |

*Identity (%) with the following reference sequences retrieved from GenBank: ST1 (U51151; human)[1] and (MK719677; human)[2]; ST2 (AB070987; human)[3]; ST3 (AB070988; human)[4], (MG807915; human)[5] and (MK719673)[6]; ST6 (AB107972; bird)[7]; ST7 (DQ232821; human)[8] and ST8 (DQ462720; non-human primate)[9].