Prevalence of *Blastocystis* and its Association with Intestinal Dysbiosis in Clinically Healthy and Metabolically Ill Subjects.

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Abstract

Background*: Blastocystis* is a typical anaerobic colon protist in humans with controversial pathogenicity and has relation with alterations in the intestinal microbiota composition (dysbiosis), whose indicator is the *Firmicutes/Bacteroidetes* ratio (*F/B* ratio); this indicator is also linked to complications such as diabetes, obesity, or inflammatory bowel disease. The present study investigated the prevalence of *Blastocystis* and its association with intestinal dysbiosis in healthy and metabolic diseased subjects.

Methods: Fecal and blood samples were collected consecutively from 200 healthy subjects, and 84 with diseased metabolic subjects; *Blastocystis* and its most frequent subtypes were identified by end-point PCR and the two most representative phyla of the intestinal microbiota *Firmicutes* and *Bacteroidetes* by real-time PCR.

Results: The prevalence of *Blastocystis* in healthy subjects was 47.0%, and 65.48 % in subjects with metabolic disease; the most prevalent subtype in the total population was ST3 (28.38 %), followed by ST1 (14.86 %), ST4, ST5, and ST7 (each one of them with 14.19 % respectively), and finally ST2 (8.78 %). The low *F/B* ratio that characterizes intestinal dysbiosis was associated with the prevalence of *Blastocystis* in the two cohorts FACSA (OR = 3.78 P <0.05) and UNEME (OR = 4.29 *p* <0.05). Regarding the subtype level, an association between the FACSA cohort ST1 and ST7 with intestinal dysbiosis was found (OR = 3.99 and 5.44 p <0.05, respectively).

Conclusions: The predatory role of *Blastocystis* over *Firmicutes* phylum is evident in both cohorts since it was observed that the abundance of the beneficial bacterial group's *Bacteroidetes* increases in the groups colonized by this eukaryote and, therefore, may have a beneficial effect**.**

Background

The population of microorganisms living in the human body, especially in the gut and its communication, is termed microbiota (1). About 10^{14} bacterial cells live in the colon besides viruses and eukaryotic microorganisms (1,2), which participate in human metabolism in an interrelated way (3). The gut microbiota plays a crucial role in maintaining the host's physiological functions, a disruption of the fragile host-microbiota interaction equilibrium could affect the onset of several metabolic diseases (4). Recently, this disruption has been implicated in some chronic diseases ranging from inflammatory bowel disease (IBD), type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD) to colorectal cancer (5).

The two predominant phyla *Firmicutes* and *Bacteroidetes* are determined mainly by the characteristics of the diet, and by some genetic and environmental factors that influence the predominance of some organisms over others (6). In case of an imbalance in the microbiota, dysbiosis will occur. The modification in *Firmicutes/Bacteroidetes* ratio is an approximate indicator of changes in the microbiota's composition, which leads to the development of long-term complications such as obesity, diabetes, or inflammatory bowel disease (7,8). Several mechanisms have been proposed to describe the intestine's

changes and energy metabolism from diets rich in carbohydrates and fats (9) that condition the gut microbiota's dysbiosis, showing a predominance *Firmicutes* phylum in individuals with obesity and type 2 diabetes mellitus (10,11).

Conversely, some parasites can colonize the human intestine that can cause changes in the typical microbiota composition. *Blastocystis* is the most frequent enteric protozoa found in the intestinal tract of humans and various animals, however, its clinical significance remains controversial (12). Infections by *Blastocystis* range from asymptomatic carriage to non-specific gastrointestinal symptoms, besides, it has been linked to irritable bowel syndrome and urticaria in some patient populations (13). This parasite can inhabit the human intestine for long periods without causing symptoms, so it can probably be part of the normal intestinal microbiota, and perhaps its pathogenic role is mainly due to the predominant subtype or the association with viruses and bacteria (14,15). Currently, 22 subtypes (STs) have been identified, 17 have been recognized, and the rest are still under investigation, in humans, only ten have been identified (ST1-ST9 and ST12) (16).

The high prevalence of *Blastocystis* infection in developing countries is usually due to poor hygiene practices, exposure to animals infected with parasites, and intake of water or food contaminated (17). The presence of the parasite increases the diversity of bacteria in the gut (18,19). A negative correlation between *Blastocystis* and body mass index (BMI) has been suggested (20), however, it is unknown whether the bacterial microbiota of these individuals induces colonization by *Blastocystis* or the presence of this protozoa promotes specific bacterial microbiota in individuals with low BMI (19,21). *Blastocystis* may cause dysbiosis and gastrointestinal symptoms, and even has been extensively studied in populations with irritable bowel syndrome and disease, the associations found are not strong enough to be attributed to the parasite (22,23). Currently, it is unknown whether *Blastocystis* participates in intestinal dysbiosis and changes in the microbiota or changes in microbiota and metabolic dysfunctions that cause high colonization by this protozoan (24). México has reported 4 to 80% of prevalence (25–28) but is unknown the association with microbiota alterations. This study aimed to evaluate the prevalence of *Blastocystis*, subtypes and its association with intestinal dysbiosis in clinically healthy and metabolically ill subjects.

Results

Two hundred two young university students (FACSA cohort) were included in the study (two were eliminated due to insufficient samples). Of 95 metabolically ill adults (UNEME cohort), 11 were eliminated due to bad samples and incomplete questionnaires. The subjects of the FACSA cohort were younger than the UNEME cohort, the median age in the groups was 20 (19-21) vs. 55 (46-63) years, respectively. The subjects of the UNEME cohort have a higher body mass index than the FACSA cohort. The percentage of subjects with normal BMI is higher in the FACSA cohort, and the urban area is the most represented in the total sample. Still, in sick adults, the rural area is more representative (Table 1).

Prevalence of *Blastocystis* and subtypes

Blastocystis was more prevalent in the UNEME cohort 55 (65.48%) vs. 94 (47.0%) p <0.05. For subtypes, ST5 was the most prevalent in UNEME cohort 16 (29.63%) p <0.05 while ST4 was more prevalent in the FACSA cohort, although the data are not statistically significant. Table 2.

Relative abundance units *Firmicutes* (RAUF), Relative abundance units *Bacteroidetes* (RAUB), and *Firmicutes/Bacteroidetes* ratio (*F/B* ratio)

The RAUF was higher in cohort UNEME 1.92 (0.71-3.53) vs. cohort FACSA 0.80 (0.05-2.08), similar results were obtained with the RAUB 1.6 (0.63-3.29) vs. 0.82 (0.44-1.65) and *F/B* ratio 1.40 (0.32-4.03) vs. 0.83 (0.07-3.50) Table 2.

Prevalence of *Blastocystis* and their association con dysbiosis intestinal

The prevalence of *Blastocystis* was associated with a lower *F/B* ratio both in the FACSA cohort and in the UNEME cohort, 0.23 (0.02-1.6) carriers vs. 1.3 (0.5-8.2) no carriers, and 0.88 (0.31-2.5) carriers vs. 2.4 (1.2-6.1) no carriers, respectively, p <0.05. The prevalence of *Blastocystis* was not associated with age, sex, or obesity in either two cohorts, Table 3.

We considered for the logistic regression analysis, a high *F/B* ratio> 0.83 and low *F/B* ratio <0.83 in the FACSA cohort, and a high *F/B* ratio> 1.40 and low *F/B* ratio <1.40 in the UNEME cohort according to the results of Table 2. The association was corroborated with respect to *Blastocystis* OR = 3.78 (95% CI 2.10-6.81) and OR = 4.24 (95% CI 1.59-11.31) p <0.05 (Table 4 and 5, respectively), ST1 and ST7 were associated with dysbiosis in the FACSA cohort but not in the UNEME cohort, ST1 has OR = 3.99 (CI 95% 1.07-14.79) and ST7 OR = 5.44 (95% CI 1.16-25.52) p <0.05, (Table 4).

Discussion

Our most important findings are the higher *Blastocystis* prevalence in the UNEME cohort than in the FACSA cohort (Table 2). The faecal-oral transmission of *Blastocystis* is due to poor hygiene practices, exposure to animals infected with the parasite, and intake of contaminated water or food (17). This transmission route is probably most common since the most UNEME cohort subjects live in rural areas (Table 1).

The *F/B* ratio comparison between cohorts showed dysbiosis in UNEME cohort subjects (Table 2) according to a high *F/B* ratio (Median of 1.40, IR: 0.32-4.03) compared to the subjects of the FACSA cohort (Median of 0.830, IR: 0.07-3.50, p <0.05). This dysbiosis agrees with previous reports, in which a high *F/B* ratio is found in metabolically ill subjects (4), this relationship implies a predisposition to disease states (29). Likewise, a low *Firmicutes/Bacteroidetes* ratio is related to weight loss (30), which corresponds with FACSA cohort individuals with lower BMI in this study (Table 1).

Obese and diabetic individuals, compared to healthy individuals, have a higher relative abundance of *Firmicutes* and a reduced abundance of *Bacteroidetes* (10), as well as a low microbial gene count and a dominance in the genera *Bacteroides* and *Ruminococcus*, all this is associated with a more remarkable

ability to obtain energy from the diet, systemic inflammation, adiposity, insulin resistance, and dyslipidemia (31,32). *Bacteroidetes* are known to produce mainly acetate and propionate, while *Firmicutes* produce more butyrate, attributed to anti-inflammatory activities, regulation of energy metabolism, and increases leptin.

The increase in *Firmicutes* in metabolically ill patients could cause an increase in the butyrate production leading to antiobesogenic effects, which is contradictory. It has been speculated that in obese subjects, the butyrate-producing bacteria decrease and are replaced by other bacteria belonging to the same phylum (33).

Blastocystis is a widely distributed organism with great adaptability that could colonize healthy and diseased subjects. We analyzed the *Firmicutes/Bacteroidetes* relationship and the presence of *Blastocystis* in these two cohorts, and the results showed an association with a low F/B ratio (Table 3). Some studies have suggested that the intestine's dysbiosis prevents colonization by anaerobic eukaryote *Blastocystis.* The decreases in butyrate available for the colonocytes' oxidative metabolism allow the increase of the luminal oxygen concentrations and, therefore, the proliferation of pathogenic bacteria. Thus, this environment would cause oxidative stress on *Blastocystis,* and therefore its survival would be affected (34,35).

Surprisingly our findings suggest a possible adaptation of *Blastocystis* to oxidative stress and low-grade inflammation that has been observed in metabolically ill patients, this low-grade inflammation was found in the UNEME cohort but not in the FACSA cohort (Table 1). Although we do not have data of interleukin expression, the data on the white formula showed higher levels of leukocytes, lymphocytes, neutrophils, and platelets in UNEME subjects, which suggest low-grade inflammation (36,37). Besides high *Blastocystis* prevalence in both cohorts, these results are unlikely caused by the protozoa. The adaptation of *Blastocystis* may have been due to its alternative oxidase mechanism (OXA) (38) and the five families of genes acquired by lateral gene transfer involved in response to oxidative stress in *Blastocystis* (39). Therefore, an essential role for *Blastocystis* is suggested in terms of this low *F/B* ratio, which may have a beneficial effect on obese individuals in the UNEME Cohort.

The relationship of *Blastocystis* with the gut microbiota is a subject of debate, as it has been linked to a low *F/B* ratio and irritable bowel disease. However, Audebert et al. (2016) found a greater abundance of Clostridiales at the class level and a greater abundance of *Rumminococcaceae* and *Prevotellaaceae* at the family level in subjects with *Blastocystis*, while *Enterobacteriaceae* increased in patients without *Blastocystis*. It has been suggested that *Blastocystis* is not associated with dysbiosis observed in intestinal, metabolic diseases or infections commonly associated with inflammation of the lower gastrointestinal tract, instead, colonization by this parasite could be associated with a healthy intestinal microbiota (18). Although still controversial, Defaye et al. (2020) reported the decrease in the *Firmicutes/Bacteroidetes* ratio ($p = 0.06$) (40) in a model of IBS (Irritable Bowel Syndrome) in rats infected with *Blastocystis* ST4 from healthy humans. This partially agrees with our findings since in the FACSA cohort ST2 had an OR = 3.12 (0.61-15.88 95% CI), ST3 OR = 1.97 (0.86-4.52 95% CI), and ST4 OR

= 2.34 (0.78-7.02 95% CI), p> 0.05 (Table 4) are not associated with Low *F/B* ratio. However, there was an association with low *F/B* at the gender level OR = 3.78 (2.10-6.81 CI 95%), for the subtypes ST1 OR = 3.99 (1.07-14.79 CI 95%) and ST7 OR = 5.44 (1.16- 25.52 95% CI) p <0.05, (Table 4). Regarding ST7, Yason et al. (2019) reported that the presence of ST7 decreases the *Bifidobacterium* and *Lactobacillus* populations while increasing the *Escherichia* populations (41).

Regarding ST1, the presence of an alternative oxidase provides a partially dependent metabolism of molecular oxygen to resist the stress that the high oxygen concentration entails, which may occur in the intestine of the FACSA subjects due to the low *F/B* ratio (39). Also, in the UNEME cohort, a low *F/B* ratio was found concerning gender OR = 4.24 p <0.05 (Table 5) but not for subtypes.

The low *F/B* ratio found in this study might imply that the subjects of the two cohorts infected by *Blastocystis* present gastrointestinal symptoms, however, the analysis of the association of the *Blastocystis* prevalence with gastrointestinal symptoms showed an inverse association between abdominal pain and ST1. At the same time, ST4 was inversely associated with abdominal distension in the FACSA cohort in previous data published (42) (Table S2 and S3 of the supplement), however, no association of *Blastocystis* with gastrointestinal symptoms was found in the UNEME cohort (Table S4 and S5 of the supplement). Contrary to the results obtained, a study found that *Blastocystis* negatively correlates with *Bacteroidetes* (18), while the phylum *Firmicutes* presents a positive correlation in *Blastocystis* positive samples (21). These results could be biased in type 2 diabetes subjects due to the low carbohydrate consumption. Since *Firmicutes* have more genes for the enzymes involved in their metabolism, a decrease in carbohydrate consumption could leads to *Firmicutes* decrease and *Bacteroidetes* increase (10,43), however, we did not analyse diet in this study.

Possibly, dysbiosis does not always lead to inflammation or disease, but certain conditions that lead to an inflammatory state would have to be present to affect the individual. For example, in obese individuals, the proportion of *Firmicutes* and *Proteobacteria* increases, compared to *Bacteroidetes*, in this case, an inflammatory environment is present (44). In the UNEME cohort, we observed dysbiosis since the *Firmicutes/Bacteroidetes* ratio increased in the UAR of *Bacteroidetes*, but this does not necessarily imply inflammation. Additionally, there is evidence that *Blastocystis* modulates the immune system through IL-22 release that stimulates the mucus production, alleviates colitis symptoms (45) and induces an immune response with a predominance of the Th2 cell response, favouring an anti-inflammatory environment (46).

Regarding ST4, although the prevalence was not high, it was more frequent in FACSA cohort (clinically healthy subjects) subjects with lower BMI (Table 1 and 2) and subjects with lower BMI in the total sample (Table S6 supplement). These findings agree with Beghini et al. (2017) that found a strong negative correlation between BMI and *Blastocystis* prevalence (21). Also, consistent with findings from the Danish subjects study (20), the difference in *Blastocystis* prevalence between average weight and obese subjects (p = 5E-03), average weight and overweight (p = 0.01), and between non-overweight and overweight (p = 0.02) was significant. Between specific subtypes, only ST4 reached statistical significance (p = 0.03

between average weight and obese). Besides, Tito et al. (2019) found a positive and significant correlation (R = 0.26 p = 0.00028) between ST4 and *Akkermansia* and *Methanobrevibacter* (19), the first is an abundant bacterium in healthy people that degrades intestinal mucin, which is associated with weight loss, the second is a *methanogenic archaeon* that plays an essential role in carbohydrate digestion and may protect against weight gain (31).

ST3 was the most prevalent in the FACSA cohort, 29.79%, and the second most prevalent, 25.93%, in the UNEME cohort (Table 2). This subtype was not associated with intestinal dysbiosis in either of the two cohorts (Table 4 and Table 5), in agreement with previous reports (48,56,57). It has been reported a higher bacterial diversity in ST3-Blastocystis-carriers (high abundance of *Prevotella*, *Methanobrevibacter*, and *Ruminococcus*), while a high percentage of *Bacteroides* found in *Blastocystis*-free subjects (57). Asnicar F. et al. (2021) reported interesting findings of the presence of *Prevotella copri* and *Blastocystis* spp as markers of improved postprandial glucose response, both were strongly linked with favourable glucose homeostasis and a decrease of the estimated visceral adipose tissue mass (58).

In future studies, we will analyse the dietary habits and the composition of their microbiota, including *Blastocystis*, in both cohorts. One of the limitations of our work was qualitative PCR, which only identifies the presence or absence of ST. The implementation of a more sensitive molecular technique, Next generation amplicon sequencing like the one used by Maloney (2019), could give us additional information, such as the most predominant ST or the existence of more than two subtypes in a single sample (59). It could also help with the identification of genotypes that were not detected with the primers used. A more accurate assessment of *Blastocystis* diversity is the key to understand the transmission mechanism and its pathogenicity in our population. Another limitation was that the majority of the subjects in the UNEME cohort were obese, and the analysis of the comparison with thin individuals between the two cohorts could not be carried out. Therefore, the objective is to increase the sample size in this cohort concerning this group.

Conclusions

The present study provides an overview of the two most representative phyla behaviour, the intestinal microbiota *Firmicutes* and *Bacteroidetes* and the *Firmicutes/Bacteroidetes* ratio when *Blastocystis* is present. The modulation caused by the parasite mainly in the *Firmicutes* phylum is evident, which decreases in two cohorts. Some studies have been reported the variability between subtypes but have not to focus on the host´s variability. Based on this, it is interesting to analyze the type of diet since this may favor the colonization and the predatory function of *Blastocystis* in the intestine, additionally, follow-up studies will be carried out in the two cohorts.

Methods

Subjects and Sample collection

This study was a cross-sectional design with a nonprobabilistic sampling conducted between March of 2018 to April 2019. Two hundred clinically healthy university young adults (FACSA cohort) and 84 adults with metabolic disease were included (UNEME cohort). Inclusion criteria for UNEME cohort were diabetes, hypertension, dyslipidemia, and overweight/obesity. Exclusion criteria were kidney failure, heart disease, lung disease, amputation, and pregnancy.

Inclusion criteria for the FACSA cohort were Bachelor students of the medicine and nutrition program. In this group, patients with chronic degenerative diseases were excluded.

Exclusion criteria for both groups were patients who received any medication with antibiotic treatment in the last three months before the study.

Data of patients with insufficient sample or incomplete information were eliminated.

Questionnaire Survey

A gastrointestinal symptoms questionnaire in digital format, consisting of multiple-choice questions, based on Rome III diagnostic criteria (60,61), was used to collect information about each participant including sex, age, city of origin, and clinical data. All data collected from each subject remained confidential and were fully anonymized through the encryption of the identity of individuals.

A 5 ml blood sample was taken of all individuals to perform hematic biometry and requested a stool sample to identify the intestinal microbiota and the presence of *Blastocystis.*

Blastocystis identification

Parasitological examination

Samples were collected in containers with 10% formaldehyde for coproparasitological exams in triplicate. Each microscopic identification of *Blastocystissp* was carried out on a different day of the deposition. The sample preparation was developed as described previously (42). Briefly the modified Ritchie technique was performed for the preparation of the samples. Ten microlitres of each stool sample was mixed with 20 µl of Lugol's iodine solution and covered with a 21 × 26 mm coverslip. Three hundred optical fields were examined for *Blastocystis* with a magnification of 250× (20× objective and 12.5 eyepieces) and, in case of suspected organisms, 500 × (40 × 12.5×) magnification. The observation of each slide lasted an average of 5 minutes. The diagnostic criterion for positivity was at least 2 precise vacuolar forms of the parasite in either of the three samples (62).

DNA extraction

To confirm the microscopy diagnosis, molecular biology techniques were used to detect *Blastocystis* subtypes. A fresh sample was dispensed into a DNase and RNAse-free sterile bottle and kept under refrigeration until transport to the laboratory, stored at -20ºC until use. The sample extraction was developed as described previously (42). Briefly from 200 mg of faeces was carried out using the

E.Z.N.A.® Stool DNA Kit (USA). DNA concentration and purity were determined using NanoDrop 1000 Thermo Scientific (Saveen Werner ApS®, Denmark).

Genus determination

The extracted DNA samples were used to determine the presence of *Blastocystis*. Three microlitres of each DNA sample were mixed with Radiant™ Red 2x Taqman Mastermix (Alkali Scientific Inc.) to a final volume of 13 ul for the PCR. The primers used were: F1- 5'-GGA GGT AGT GAC AATAAA TC-3' and R1- 5'-CGT TCA TGA TGA ACA ATT AC-3' (T4 Oligo®, Irapuato, México).

Subtyping of *Blastocystis* using Sequence-Tagged Sites (STS) primers

For the genotyping of *Blastocystis*, a set of sequence-tagged site primers derived from products of randomly amplified polymorphic DNA (RAPD) sequences were used (63,64). Four ul of each DNA sample positive for *Blastocystis* in a Polymerase chain reaction (PCR) was mixed with Radiant™ Red 2x Taqman Mastermix (Alkali Scientific Inc.) with primers (Table S1) in a final volume of 13 ul.

The PCR conditions were an initial denaturation step at 94°C for four minutes, followed by 35 denaturation cycles at 94°C for 30 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 10 minutes (PTC-100 thermocycler, MJ Research Inc) [30]. The B-globin gene was amplified as an internal extraction control. The PCR products were resolved in a 1.5% agarose gel (Ultrapure Agarose, Invitrogen™) stained with RedGel™ Nucleic Acid Gel Stain (Biotium) and a molecular weight marker was used to establish the size of the amplicon (100 bp DNA Ladder. Biobasic Inc.). Additionally, the samples were randomized to the analysis by PCR. Sanger sequencing was used to corroborate both the presence of *Blastocystis* and genotypes.

Identification of the gut microbiota

The analysis of the microbiota profile was performed by real-time PCR (qPCR) using the 16S rRNA gene-specific and universal primers. The sequences of the primers were: *Bacteroidetes*: AAACTCAAAKGAATTGACGG (Forward) and GGTAAGGTTCCTCGGCTAT (Reverse), *Firmicutes*: TGAAACTYAAGGAATTGACG (Forward) and ACCATGCACCACCTGTC (Reverse), universal: AAACTCAAAKGAATTGACGG (Forward) and CTCACRRCACGAGCTGAC (Reverse) (T4 Oligo®, Irapuato, México).

To each PCR reaction, 5 ul of SYBR Green (Maxima SYBR Green qPCR Master Mix, Thermofisher Scientific TM), 1 ul of each primer (concentration of 5 pmol for Reverse and 10 pmol for Forward), 1 ul of DNA, and 2 ul of DNase/RNase-free water were added to a final volume of 10 ul. Each reaction was performed in duplicate.

The analysis of the qPCR amplification was performed with the Rotor-Gene Q equipment (QIAGEN®, Germany). The samples were processed under the following amplification conditions: an initial thermal denaturation cycle of five minutes at 95°C, alignment with 30 cycles at 59°C for 15 seconds and

elongation for 20 seconds at 72°C. The conditions were the same for the three pairs of primers used (Universal, *Bacteroidetes* and *Firmicutes*). The expression analysis was carried out by quantifying the relative abundance units (RAU) of *Firmicutes* and *Bacteroidetes* with the formula RAU= $2^{-\Delta Ct}$ where: RAU = Relative Abundance Units and ΔCt = Ct specific primers-Ct universal primers (65).

Hematic Biometry

The quality control was performed through 3 controls: low, normal, and high (KX-21N SYSMEX LOT: 2R0301) in the automated KX-21N equipment. Each blood differential of a complete red series and white series grouping (Lymphocytes, neutrophils, and the sum of basophils and eosinophils monocytes (MXD)) were performed.

Statistical analysis

The Kolmogorov-Smirnov normality test was used. Non-parametric U-Mann-Whitney tests were used for comparisons between the medians of the two groups. The *Firmicutes/Bacteroidetes* ratio variable was operationalized by taking the cut-off point above and below the median of the RAU on each phylum. For the bivariate analysis, a chi-square test (x^2) or Fisher's exact test were applied. The odds ratio (OR) and 95% confidence interval were estimated. A *P* value <0.05 was considered significant. The statistical analysis was performed using the Stata® Statistics Package, version 13.0.

Abbreviations

RAU: Relative abundance units, RAUF: Relative abundance units of *Firmicutes*, RAUB: Relative abundance units of *Bacteroidetes*, ratio *F/B*: ratio *Firmicutes/Bacteroidetes*, IBD: Inflammatory bowel disease, T2D: Type 2 diabetes, CVD: cardiovascular disease, BMI: body mass index, FACSA: Facultad de Ciencias de la Salud, UNEME: Unidades en especialidades médicas, UNEME-EC: Unidades en especialidades médicas en enfermedades crónicas, mm^3: cubic milimetre, mg/dL: milligrams per deciliter, mt^2: square meter, DNase: Deoxyribonuclease, RNase: Ribonuclease, USA: United States of America , RAPD: Random Amplified Polymorphic acid deoxyribonucleic, PCR: Polymerase Chain Reaction, qPCR: Quantitative polymerase chain reaction, rRNA:Ribosomal ribonucleic acid, ST: Subtype 1, ST2: Subtype 2 ,ST3: Subtype 3, ST4: Subtype 4, ST5: Subtype 5, ST7: Subtype 7, IR: interquartile range, OR: Odds Ratio, CI 95 %: Confidence interval to 95%, n: number, %: percentage. CHS: Colonic Hypersensitivity: SCFAs: Short-Chain-Fatty-Acids, AOX: Alternativa oxidasa, MXD: Sum of Basophils eosinophils and monocytes.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Faculty of Health Sciences of the Juárez University of the State of Durango (PI-01-2018). Written informed consent was obtained from the subjects included in this study.

Consent for publication

Not applicable

Availability of data and materials

The nucleotide sequences generated in present study have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/) under accession numbers: BankIt2463554, BankIt2463557, BankIt2464109, BankIt2464110, BankIt2464116, BankIt2464118 and BankIt2464119.

Competing interests

The authors declare that they have no competing interests.

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Authors`Contributions

JOGG and CMY conceived and designed the study and wrote the paper. Faecal samples were collected and analysed by CMY and ALMH. Sociodemographic and anthropometric data were collected by AMS, MAMD and SAZM. Data were analysed by JOGG, MAMD and SAZM. All authors have read and approved the submitted version of this manuscript.

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Tables

Table 1. Characterization of FACSA and UNEME cohort.

	Total	FACSA	UNEME
Age, years	21 (19-45)	20 (19-21)	55 (46-63) *
Sex, n(%)			
Female	201 (70.77)	132 (66.0)	69 (82.14) *
Male	83 (29.23)	68 (34.0)	15 (17.86)
BMI, kg/mt2	26.60 (22.39-31.4)	24.29 (21.08-28.76)	31.55 (28-35.50) *
Normal	118(41.55)	111(55.50)	7 (8.33)*
Sob/obesity	166(58.45)	89(44.50)	77(91.67)
Place of residence			
Urban	197 (69.37)	170 (85.0)	27 (32.14)
Rural	87 (30.63)	30 (15.0)	57 (67.86) *
Hematic Biometry			
Leukocytess/mm3	6.91 ± 1.67	6.61 ± 1.60	7.57 ± 1.63 *
Total Lymphocytes/mm3	2.2 (1.8-2.6)	2.1 (1.7-2.5)	2.3 (1.9-2.7) *
Total MXD /mm3	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.5 (0.3-0.7) *
Total Neutrophils /mm3	4.10 ± 1.3	3.92 ± 1.26	4.65 ± 1.31 *
Platelets/mm3	257.44 ± 57.95	250.84 ± 51.64	272.44 ± 68.1 *

Table 2. Prevalence of *Blastocystis* and subtypes, gut intestinal in cohorte FACSA and cohorte UNEME.

	Total	FACSA	UNEME
***Blastocystis*, n(%).**	153 (52.46)	94 (47.0)	55 (65.48) *
Subtype 1 (ST1)	22 (14.86)	14 (14.89)	8 (14.81)
Subtype 2 (ST2)	14 (8.78)	8 (8.51)	5 (9.26)
Subtype 3 (ST3)	42 (28.38)	28 (29.79)	14 (25.93)
Subtype 4(ST4)	21 (14.19)	16 (17.02)	5 (9.26)
Subtype 5 (ST5)	21 (14.19)	5 (5.32)	16 (29.63) *
Subtype 7 (ST7)	21 (14.19)	12 (12.77)	9 (16.67)
Gut microbiota			
RAUF, Median IR	1.07 (0.23-2.35)	0.80 (0.05-2.08)	1.92 (0.71-3.53) *
RAUB, Median IR	0.96 (0.47-2.11)	0.82 (0.44-1.65)	1.16 (0.63-3.29) *
F/B ratio, Median IR	0.95 (0.22-3.50)	0.83 (0.07-3.50)	1.40 (0.32-4.03) *

Table 3. Prevalence of *Blastocystis* and its association with age, sex, and gut microbiota in FACSA and UNEME cohort.

	FACSA *Blastocystis*		*UNEME* *Blastocystis*	
	no carriers	carriers	no carriers	carriers
Age, median IR	20 (18-21)	20 (19-21)	52 (47-61)	57 (46-64)
Sex, n(%)				
Male	35 (51.4)	33 (48.5)	6 (40.0)	9 (60.0)
Female	71 (53.7)	61 (46.2)	23(33.3)	46 (66.6)
Normal	60 (54.0)	51 (45.9)	1(14.2)	6 (85.7)
Sob/obesity	46 (51.6)	43 (48.3)	29 (34.5)	49 (63.6)
Microbiota				
RAUF	1.4 (0.80-2.8)	0.26 (0.05-0.7)*	2.1 (1.2-4.9)	1.7 (0.63-2.2)
RAUB	0.88(0.28-2.1)	0.78(0.47-1.3)	1.06(0.35-1.9)	1.2(0.65-3.6)
Ratio F/B	1.3(0.5-8.2)	0.23(0.02-1.6)*	2.4(1.2-6.1)	0.88(0.31-2.5)*

Table 4. *Blastocystis*, subtypes and their association with intestinal dysbiosis in FACSA cohort.

	OR	CI 95 %	*P-value*
Blastocystis	3.78	(2.10-6.81)	**0.00**
ST1	3.99	(1.07-14.79)	**0.03**
ST2	3.12	(0.61-15.88)	0.16
ST3	1.97	(0.86-4.52)	0.10
ST4	2.34	(0.78-7.02)	0.12
ST5	4.12	(0.45-37.57)	0.20
ST7	5.44	(1.16-25.52)	**0.03**

OR: Odds Ratio; CI 95 %: Confidence interval to 95 %. $p<0.05$

Table 5. Logistic regression between *Blastocystis* and subtypes and its association with intestinal dysbiosis in a UNEME cohort.

	OR	IC 95 %	*P-value*
Blastocystis	**4.24**	**(1.59-11.31)**	**0.00**
ST1	0.58	(0.13-2.62)	0.48
ST2	1.57	(0.24-9.97)	0.62
ST3	3.06	(0.87-10.72)	0.08
ST4	0.23	(0.02-2.22)	0.20
ST5	2.71	(0.84-8.66)	0.09
ST7	2.22	(0.51-9.58)	0.28

OR: Odds Ratio; CI 95 %: Confidence interval to 95 %. $p<0.05$

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