ORIGINAL ARTICLE

Gut Microbiota and Its Associations With Lifestyle Factors in Bipolar and Major Depressive Disorders: A Preliminary Analysis

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ABSTRACT

Introduction: Gut dysbiosis is a potential aetiology of bipolar disorder (BD) and major depressive disorder (MDD), though its links to sleep duration, physical activities, and fibre intake remain poorly understood. **Materials and method:** A total of 30 faecal samples from BD, MDD, and healthy control (HC) groups, were profiled using the 16s rRNA gene sequencing technique, which their relative abundance results were further correlated with sleep duration, physical activities, and fibre intake via Pearson's correlation coefficient. **Results:** BD and MDD groups exhibited higher Bacteroidota but lower Firmicutes abundance than HC at the phylum level. In contrast, at the genus level, *Bacteroides* was most abundant in MDD, *Prevotella* in BD, and *Faecalibacterium* in HC. The alpha and beta diversity showed no significant differences across groups. However, the LEfSe analysis identified three taxa at the order level inclusive of Oscillospirales (LDA score: 2.53; p-value: 0.07) and Coriobacteriales (LDA score: 2.19; p-value: 0.03) abundance in HC, while Acidaminococcales (LDA score: 2.16; p-value: 0.07) abundance in MDD. Despite low correlations, sleep duration show two significant associations with the profiled gut microbiota: *Parabacteroides* (r-value: -0.396; p-value: 0.03) and *Alloprevotella* (r-value: 0.370; p-value: 0.04). However, associations with fibre intake and physical activities were not significant. **Conclusion:** These preliminary findings suggest that gut dysbiosis is present in both BD and MDD compared to healthy controls, with potential associations with sleep duration. These results highlight the importance of further validation in future studies to explore the mechanism underlying their relationship and their clinical implications.
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INTRODUCTION

Bipolar disorder (BD) and major depressive disorder (MDD) are both mental health conditions that underlie affective disorders (1). MDD is characterised by a person enduring a significantly low mood and the inability to feel interest or joy for a prolonged time, whereas BD is characterised by the presence of elevated mood, either mania or hypomania, with or without one depressive episode (2). However, individuals with BD are more likely to experience depressive episodes that last longer, causing episodes of mania or hypomania to be frequently undetected. This oversight often results in delays in diagnosis or misdiagnosis, such as MDD (3).

Over time, the number of people diagnosed with BD and MDD has increased significantly, with approximately 40 million and 280 million cases worldwide, respectively (4, 5). Mental health, including BD and MDD, was ranked as the seventh highest contributor to Disability-Adjusted Life Years (DALYs) globally in 2019, reflecting

its substantial burden (6). In Malaysia, although the exact figures for BD and MDD patients are not available, the prevalence of mental disorders has nearly tripled, rising from 10.7% in 1996 to 29.2% in 2015, with mood disorders being the most prevalent, as reported in the National Health Morbidity Survey (7). Alarmingly, depressive disorder contributes to 6.9% and ranked ninth for DALYs in 2019 (8, 9). These statistics underscore the impact of mental disorders on individuals' quality of life, as these conditions often persist, recur and affect their psychosocial well-being (10).

Therefore, numerous continuous studies investigating the aetiology of both disorders were conducted, including the effects of lifestyle factors, neurotransmitters, and gut microbiota on mental health; however, their findings remain inconclusive (11, 12). Besides, despite the longstanding recognition of lifestyle factors, especially sleep, which is also one of the diagnostic indicators and treatment, such as wake therapy, they are often overlooked or underutilised in clinical practice, highlighting gaps between theory and clinical applications (13). Therefore, an increasing interest in investigating lifestyle factors such as sleep, physical activity, diet and smoking with the correlation between mental health, including mood disorders, was observed (11).

Individuals with BD and MDD often experience sleep disturbance and may experience conditions like sleeping pattern disruption, hypersomnia and insomnia. This phenomenon can result in difficulties maintaining focus and delayed responses over time (14). Additionally, as they tend to lead to more sedentary lifestyles, the risk of other health issues such as cardiovascular diseases, cancer, and obesity is higher (15). Furthermore, the current treatments for BD are heavily reliant on drugs, and certain medications may aggravate health risks (16). For instance, the use of atypical antipsychotics such as olanzapine in BD patients may lead to weight gain and insulin resistance. In addition, these medications with poor diet habits and a sedentary lifestyle may further aggravate the patients' health towards developing type 2 diabetes mellitus and cardiovascular diseases (17). Besides, lower dietary fibre intake among individuals with BD and MDD has been linked to heightened risk and symptoms of depression (11, 18).

While investigating the lifestyle factors, the association of gut microbiota with mental disorders has slowly come to light (19). Gut microbiota releases biochemical compounds essential for regulating the body's homeostasis, including short-chain fatty acids and amino acids (20). Consequently, changes in gut microbiota composition, known as gut dysbiosis, can lead to various diseases, including mental disorders due to the imbalanced production of various microbial metabolites and immune mediators from the gut microbiota, causing alterations in neurotransmission, promoting neuroinflammation and altering behaviour (12). For example, *Faecalibacterium* was associated with enhanced sleep quality and reduced depressive symptoms in BD patients. This bacterium was known to release butyrate, sleeping-enhancing properties (19).

However, research on the association between gut dysbiosis and BD and MDD through lifestyle factors remains limited, particularly in human clinical studies, despite its significant potential (11, 19). Moreover, a review by Yan et al., mentioned that existing studies failed to capture the relationship between gut microbiota and the sleep factor, and there are only two studies on the association of gut microbiota with fibre supplementation in mental health (19). Therefore, this study aimed to examine gut microbiota composition and elucidate their relationship with sleep duration, physical activities and fibre intake in individuals with BD and MDD compared to healthy controls (HC) in a Malaysian setting. The findings from this research could provide insights into the aetiology of BD and MDD in relation to gut microbiota and lifestyle factors, potentially leading to enhancements in the current methods for diagnosis and treatment.

MATERIAL AND METHODS

A cross-sectional study was designed to investigate the possible aetiology of bipolar disorder (BD) and major depressive disorder (MDD) through the perspective mechanism of gut microbiota and their association with lifestyle factors. For comparison purposes, the gut microbiota composition of BD and MDD were compared to healthy control (HC) group. Their eligibility was assessed according to the inclusion criteria prior recruiting as the study subjects. A written consent was obtained upon recruitment, followed by a simple sociodemographic and clinical background check. The assessment of lifestyle factors was divided into three parts: sleep duration, physical activities, and dietary fibre intake. Faecal samples were collected for gut microbiota composition assessment and were further correlated with all three lifestyle factors. The methodology for this study is summarised in Figure 1.

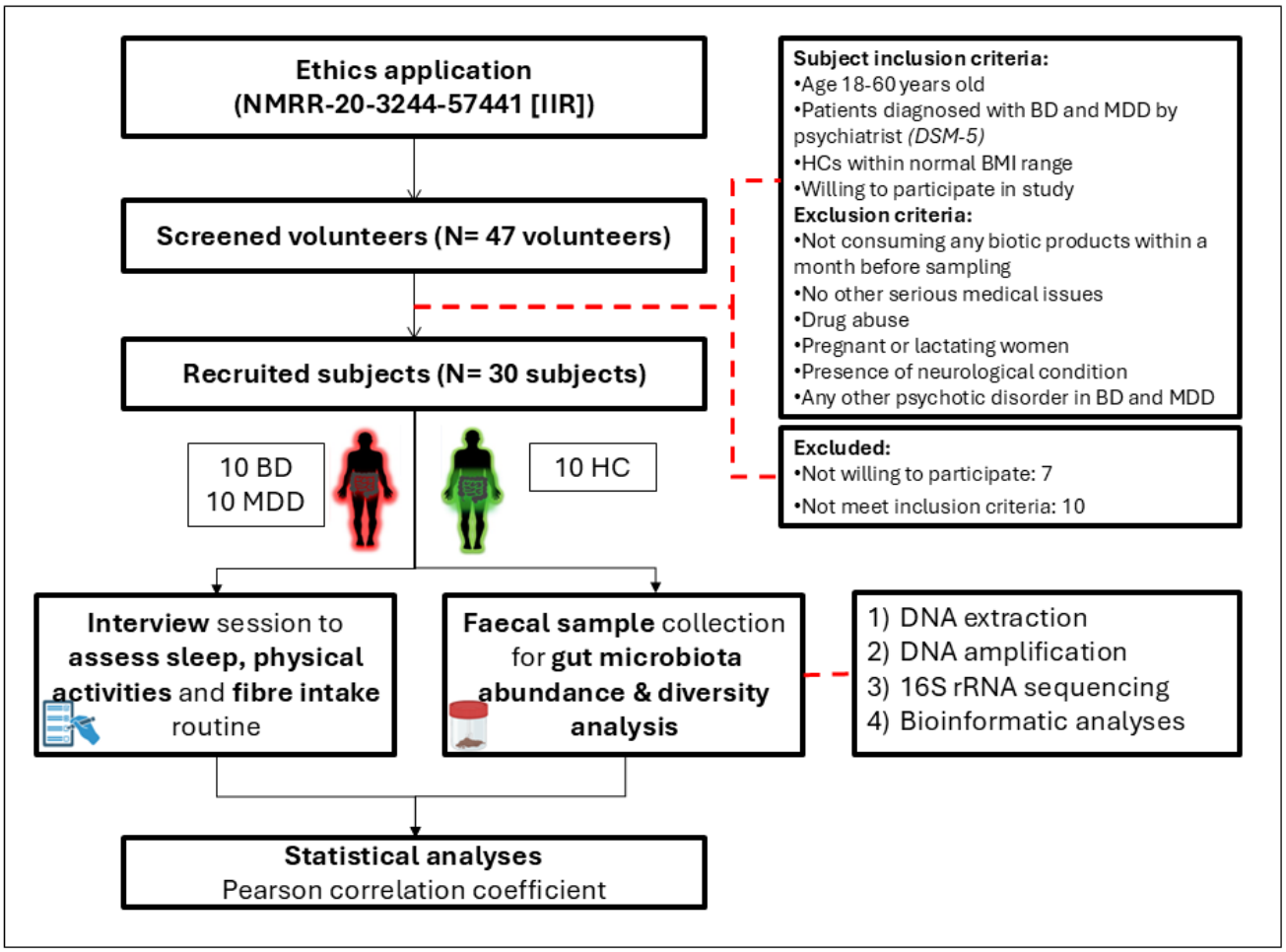

Figure 1: Overview for the research methodology

Population

This cross-sectional study was designed to determine the gut microbiota composition and their association with sleep duration, physical activities and fibre intake of BD and MDD patients compared to healthy controls (HC). The human ethics approval was obtained from the Malaysian Medical Research Ethics Committee (MREC) (NMRR-20-3244-57441 [IIR]) for three years, from April 2021 until June 2024. According to the inclusion criteria, 30 individuals were recruited, with 10 individuals for each group. BD and MDD patients were recruited from the outpatient Department of Psychiatry at Hospital Kuala Lumpur (HKL) and Hospital Angkatan Tentera Tuanku Mizan (HATTM), while healthy individuals were not restricted. Potential participants were briefed on the studies and, if interested, were subsequently screened for eligibility.

The general inclusion criteria were individuals with: (i) age between 18-60 years old, (ii) BD and MDD patients diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders V (DSM-V) and (iii) HC participants must be within the normal BMI range, reported no psychotropic medication and denial of significant mood symptoms during screening interview session. Meanwhile, the exclusion criteria were as follows: (i) consuming any biotics within three months prior to sampling, (ii) current drug or substance abuse or dependence, (iii) the presence of serious or unstable medical diseases, (iv) pregnant or lactating women, (v) presence of neurological condition, (vi) diagnosis of any other psychotic disorder for BD and MDD patients.

Assessment of sociodemographic and clinical background

Before enrolment, participants were briefed on the detailed study protocol, and written informed consent was obtained upon agreement. Respondents were then given a set of questionnaires for sociodemographic background, which included age, gender, and ethnicity. Clinical characteristics, inclusive of smoking status and medical history, including medication and supplements, were also recorded through the questionnaire. For anthropometric measurement, the body weight of each participant was measured using a digital weighing balance. BMI measurements were further carried out by dividing the square of height in meters with weight in kilograms. The obesity classification was grouped into four based on Asia-Pacific guidelines as follows: (i) underweight < 18.5 kg/m2, (ii) normal: 18.5-22.9 kg/m2, (iii) overweight: 23-24.9 kg/m2 and (iv) obese ≥ 25 kg/m2 (20). Depressive symptom severity was assessed

using the 17-item Hamilton Rating Scale for Depression (HAMD) for both BD and MDD patients, while the Young Mania Rating Scale (YMRS) scale was used to assess symptoms severity of hypomania or mania in BD patients. The rating score for HAMD ranges from; (i) 0-7: normal, (ii) 8-13: mild depression, (iii) 14-18: moderate depression, (iv) 19-22: severe depression, (v) ≥ 23: very severe depression (21).

Assessment of sleep duration, physical activities and fibre intake

Each participant was interviewed to evaluate their routines for sleep duration, physical activities, and fibre intake over the past seven days. The interview included a series of multiple-choice questions adapted from two validated questionnaires: the Pittsburgh Sleep Quality Index (PSQI) for assessing sleep routines and the Simple Lifestyle Indicator Questionnaire (SLIQ) for evaluating physical activities and fibre intake (22, 23). The average sleep duration per day over the past week was categorised into four groups: (i) less than five hours, (ii) 5-6 hours, (iii) 6-7 hours, and (iv) more than seven hours. Physical activities and fibre intake frequencies were measured on a scale ranging from zero, 1-3 times per week, 4-6 times per week, to seven days per week. The interviews were conducted in either Malay or English, depending on the participant's language preference and understanding.

Faecal sample collection and DNA extraction

The participant collected the faecal sample within one week of recruitment using a sterilised collection kit and kept it at 4˚C while waiting for collection by research personnel. The sample was delivered to the laboratory in an icebox within two hours of collection and then promptly stored at -20˚C until DNA extraction. Total genomic DNA was extracted from 200 mg of the faecal sample according to the manufacturer's protocol, using a QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany). The extracted DNA was quantified using the NanoDrop spectrophotometer. The integrity of extracted genomic DNA was assessed by 1.0 % agarose gel electrophoresis. The extracted DNA was stored at -20 ˚C in TE buffer prior to usage in 16S rRNA amplification and sequencing.

16S rRNA amplification and sequencing

The hypervariable V3-V4 region of the prokaryotic 16S rRNA gene was amplified using the primers described by Klindworth et al. (24). The forward primer was constructed with the Illumina 5' overhang adapter sequence (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG) followed by the forward primer sequence (CCTACGGGNGGCWGCAG). The reverse primer was constructed with another 5' overhang adapter sequence (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG) followed by the reverse primer sequence (GACTACHVGGGTATCTAATCC).

Amplifications were performed in 25 uL reactions using 12.5 uL of KAPA Hifi HotStart ReadyMix (KAPA Biosystems, USA), 5 uL of each 1 uM primer, and 2.5 uL of DNA template. The thermal profile was as follows: 95°C for 3 minutes, then 25 cycles of 95°C for 30 seconds, 53°C for 30 seconds, 72°C for 30 seconds, followed by one cycle of 72°C for a minute and 4°C hold. The amplification products were cleaned using Agencourt AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA).

The second round of amplification was performed to incorporate the Illumina i5 and i7 adapters and 8bp barcodes. The reaction was performed in a 10 uL reaction with 5 uL of KAPA Hifi HotStart ReadyMix (KAPA Biosystems), 1 uL of Nextera XT Index 1 primer, 1 uL of Nextera XT Index 2 primer, and 3 uL of product from first round of amplification. The thermal profile was as follows: 95°C for 3 minutes, then eight cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, followed by one cycle of 72°C for a minute and 4°C hold.

The final libraries were cleaned up using Agencourt AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using a Qubit 2.0 fluorometer (Invitrogen, CA, USA). The size distribution of the libraries was assessed using TapeStation 2200 (Agilent Technologies, CA, USA). All libraries were pooled in equimolar and sequenced on the Illumina MiSeq (Illumina, San Diego, CA, USA) at Monash University Malaysia Genomics Platform, using a 2 × 250 bp run setup (25).

Bioinformatic analysis

The raw FASTq files were processed using the DADA2 pipeline by trimming the sequence, paired-end merging and chimaera removal to generate amplicon sequence variants (ASVs). The filtering process applied the standard trimming parameters to remove the first 15 nucleotides and minimise primer effects. Taxonomic assignment and the creation of the observation table, which was rarefied at 8,000 reads, were carried out using RDP Classifier 2.12 trained on the SILVA 16S sequence database. The final sequence data was converted into a phyloseq object encompassing a basic table, including a taxonomy table, metadata files, and an ASVs table. It was subsequently used for downstream bioinformatics analyses.

The analyses of relative abundance, alpha and beta diversity, and LEfSe were performed to observe the presence of gut dysbiosis in BD and MDD individuals compared to HC by using the MicrobiomeAnalyst 2.0 online platform using the Marker-Gene Data Profiling (MDP) module (26, 27). Data normalisations were performed on the rarefied data using cumulative sum scaling (CSS). The alpha diversity was measured using Simpson, Shannon and Chao1 indices, while the beta diversity was calculated using the Bray-Curtis distance to account for compositionality. The LEfSe analysis was

conducted at the order level with an LDA threshold score of 2.0.

Statistical analysis

Statistical analysis was performed using an SPSS software version 20 (SPSS, Chicago, IL, USA). Datasets were assessed for normal distribution using the Shapiro-Wilk test, skewness, and kurtosis. Sleep duration, physical activities, and fibre intake data were analysed as categorical, while data related to the gut microbiome was analysed as continuous. Normally distributed continuous data was presented as mean ± SD (standard deviation), while not normally distributed continuous data was presented as median and interquartile range. Categorical data was presented as frequency (n) and percentage (%). Statistical analyses of gut microbiota profiling were carried out using the MicrobiomeAnalyst. Correlation analyses between sleep duration, physical activities and fibre intake with gut microbiota composition were conducted using the Pearson's correlation coefficient method in RStudio v4.0 employing the cor() function and visualised with the corrplot package. The correlation strength was indicated through R-values, which were divided into five categories: very low (r-value: 0.000-0.199), low (r-value: 0.200-0.399), moderate (r-value: 0.400-0.599), high (r-value: 0.600-0.799) and very high (r-value: 0.800-1.000). Data was considered statistically significant if the *p-value <0.05, **p-value <0.005, ***p-value <0.001.

RESULTS

Sociodemographic and clinical characteristics of the population

The characteristics of the participants are summarised in Table I. Thirty individuals were recruited and grouped based on their health category: 10 BD participants, 10 MDD participants and 10 HC participants. Participants from BD and MDD were adults with a median age of 39 and 40 years old, respectively, while the HC were young adults with a median of 21 years old. The majority of the participants are of Malay ethnicity. The majority of BD and MDD subjects were obese and overweight, respectively. Participants from these two groups are primarily females and had higher smoking status compared to HC. About 80-90% of BD and MDD subjects, respectively, were on medications. The common medications included escitalopram and sertraline for MDD, while for BD were epilim and lithium.

Table I: Demographic characteristics and clinical data for recruited participant

Characteristics	Bipolar Disorder (N=10)	Major Depressive Disorder (N=10)	Healthy Control (N=10)	p-value
Age (years)	39.00 (6.75)	40.00 (14.25)	21.00 (0.25)	0.000
BMI (kg/m^2)	30.48 (3.98)	23.58 (11.74)	23.31 (5.81)	0.004
Gender [n (%)]				0.272
Female	6(60)	7(70)	3(30)	
Male	4(40)	3(30)	7(70)	
Ethnicity [n (%)]				0.507
Malay	10(100)	8(80)	8(80)	
Other	0(0)	2(20)	2(20)	
Medication				1.000
Yes	9(90)	8(80)	-	-
No	1(10)	2(20)	-	-
Smoking Status [n (%)]				0.195
Yes	3(30)	3(30)	0(0)	
No	7(70)	7(70)	10(100)	
HAMD score	17 (19)	21 (9.75)	-	0.902
YMRS score	4.8 ± 2.02	-	-	
Sleep [n (%)]				0.510
< 6 hour/day	5(50)	6(60)	8(80)	
> 6 hour/day	5(50)	4(40)	2(20)	
Physical activity [n (%)]				0.008
None	4(40)	4(40)	0(0)	
1-3 times/week	6(60)	5(50)	4(40)	
4-7 times/week	0(0)	1(10)	6(60)	
Fibre intake[n (%)]				1.000
0-3 times/week	2(20)	3(30)	3(30)	
4-7 times/week	8(80)	7(70)	7(70)	

Data were presented as median (interquartile range) or mean ± standard deviation for continuous data while n (%) for categorical data. P-values were calculated using a Kruskal-Wallis's test and Chi-Square test.

Meanwhile, for the HAMD score, the MDD group showed a significantly higher score, indicating a more severe depression state compared to the BD group. The median HAMD score for MDD and BD was 21 and 17, respectively. In addition, when paired with scores of the YMRS at 4.8, the BD group would be deemed to be in a euthymic episode in which, for the BD patients, a HAMD score of <14 and a YMRS score of <9 was achieved (28). However, as the median HAMD score was 17, the BD group was experiencing moderate depression phase.

Sleep duration, physical activities and fibre intake assessments

The scores for sleep duration, physical activities, and fibre intake for the past week are tabulated in Table I. The HC groups had slept less than six hours, whereas the BD and MDD group had similar short and long sleep duration. Regarding their physical activities, the BD and MDD groups significantly spent less than four times a week on physical activities compared to the HC group. Regarding fibre intake, all groups consumed their fibre regularly throughout the week.

Gut microbial relative abundance

All three groups had relatively similar abundance of gut microbiota composition, as shown in Figure 2 and Supplementary Table I. The phyla level was dominated by four types of bacterial taxa: the Bacteroidota, Firmicutes, Proteobacteria and Actinobacteriota (Figure 2A). However, it was observed that the BD and MDD groups had higher levels of Bacteroidota and lower levels of Firmicutes than the HC group (Supplementary Table I). At the genus level, *Bacteroides*, *Prevotella* and *Faecalibacterium* have dominated the microbiota populations of each subject group (Figure 2B). *Bacteroides* was the most abundantly found in the MDD, *Prevotella* in the BD, and *Faecalibacterium* high in the HC.

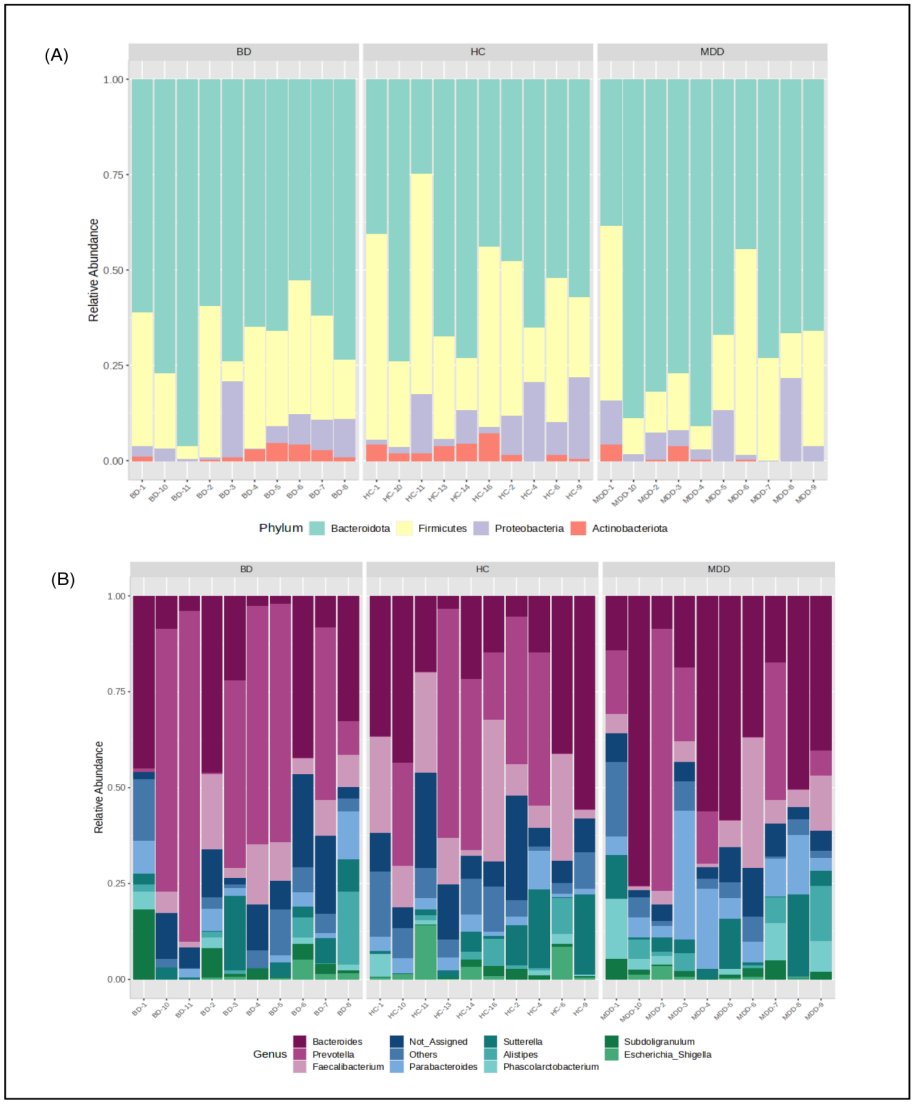

Figure 2: (A) Four relative abundance of gut microbiota at the phylum level for each group. (B) Top 10 relative abundance of gut microbiota at the genus level for each group.

Gut microbiota diversity

Alpha diversity of gut microbiota composition was measured using the Shannon index, Simpson index and Chao1 index. Based on Figure 3(A), the HC group was observed to have the highest species richness and evenness of the gut microbiota composition compared to the BD and MDD groups. Next, in Figure 3(B), compared to BD and MDD, the HC had the highest Simpson index value, indicating greater species evenness, which also suggested lower species diversity. Lastly, the Chao1 index Figure 3(C) estimated that the BD had the highest index value compared to the HC and MDD. Nevertheless, these findings are not statistically significant, with p-values of 0.473, 0.295 and 0.798, respectively.

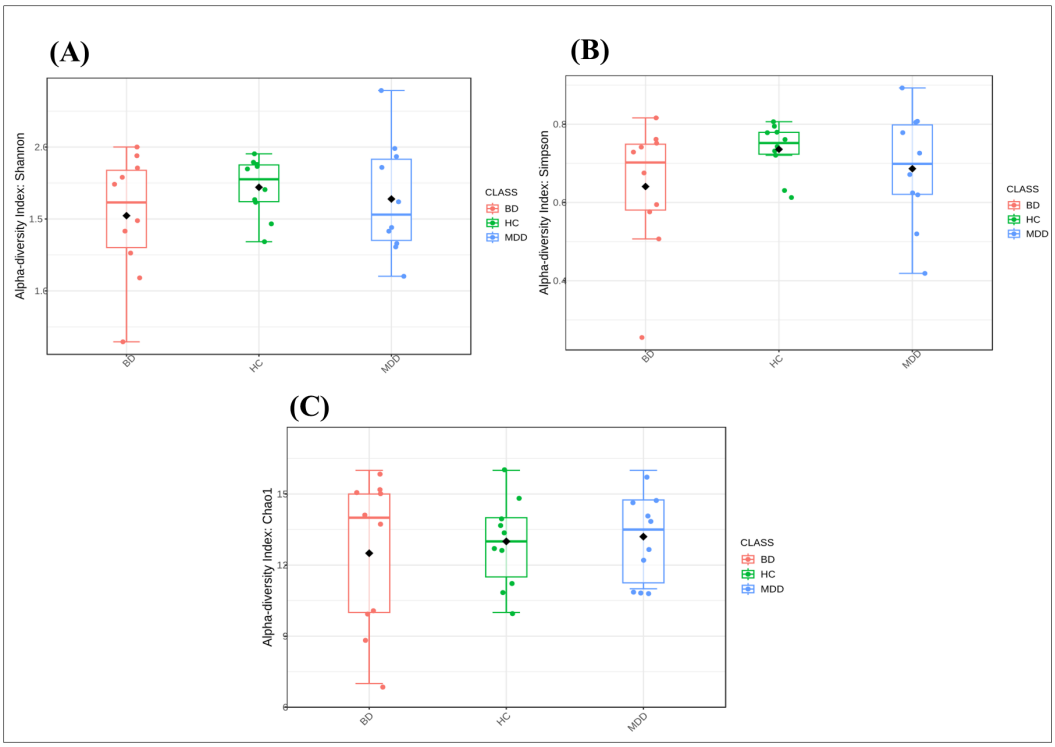

Figure 3: Boxplot of alpha diversity. (A) Shannon index (B) Simpson index (C) Chao1 index.

An overlapping ordination pattern was observed in the beta diversity analysis using the Bray-Curtis (BC) distance; hence, there were no significant differences in the gut microbiota composition for all three groups (Figure 4). The PCoA analysis also revealed that the statistical value was not significant, with a p-value of 0.167.

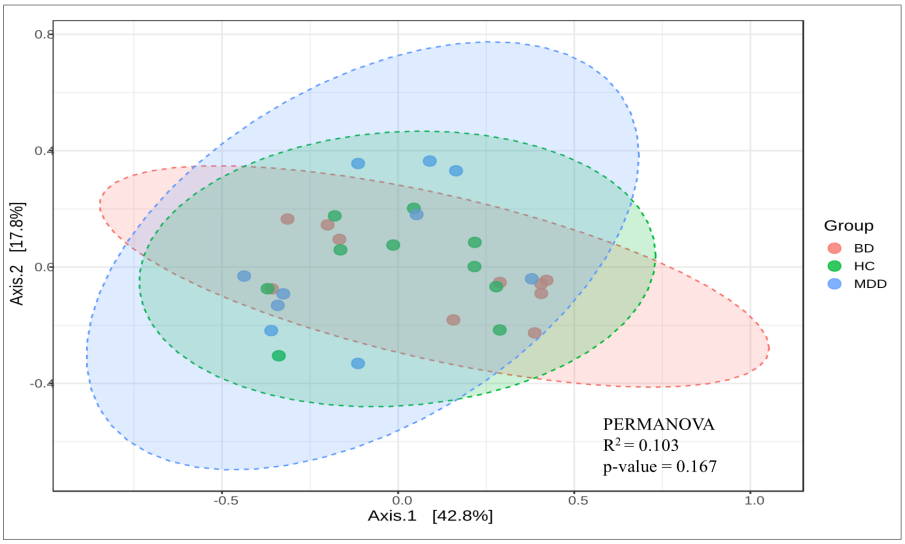

Figure 4: Beta diversity based on the BC distances of the gut microbiota for three groups.

Differential taxonomic compositions of gut microbiota in the BD, MDD and HC groups

In the LEfSe analysis, only three bacterial taxa at the order level were significantly abundant, where two taxa were from the HC, and another was from the MDD group (Figure 5). At the order level, Oscillospirales (LDA score: 2.53, p-value: 0.07) and Coriobacteriales (LDA score: 2.19; p-value: 0.03) were dominant in HC, while Acidaminococcales was dominant in the MDD (LDA score: 2.16; p-value: 0.07). However, after FDR correction, no significant difference in gut microbiota at all levels was discovered for all three group.

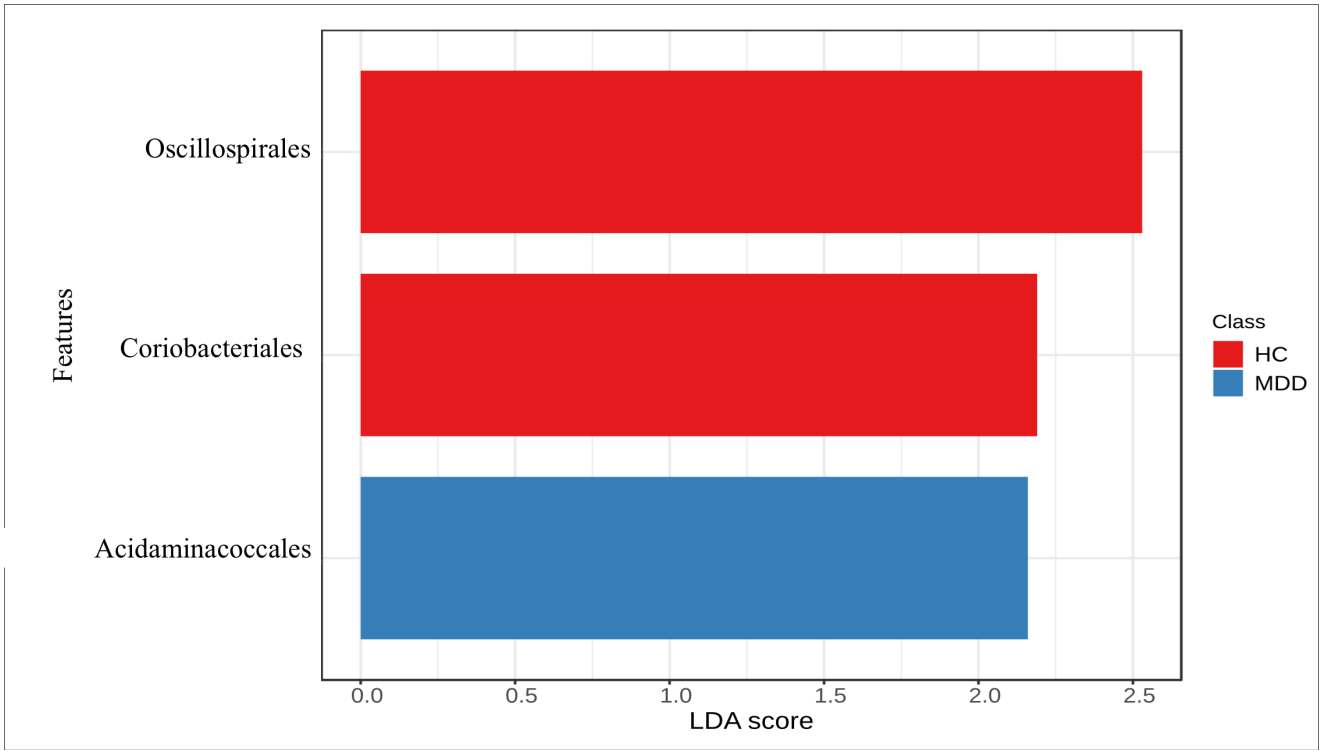

Figure 5: Differential gut microbiota taxa down to order level between HC and MDD groups

Correlation of gut microbiota with sleep duration, physical activities and fibre intake

The correlation between the top 10 genus and lifestyle factors (sleep duration, physical activities and fibre intake) was determined using the Pearson correlation analysis. According to Figure 6, only sleep duration significantly correlates with the gut microbiota, *Parabacteroides* (r-value: -0.396; p-value: 0.03) and *Alloprevotella* (r-value: 0.370; p-value: 0.04). However, both correlations only indicated a low correlation' strength. All the correlations involved are summarised in Supplementary Table II.

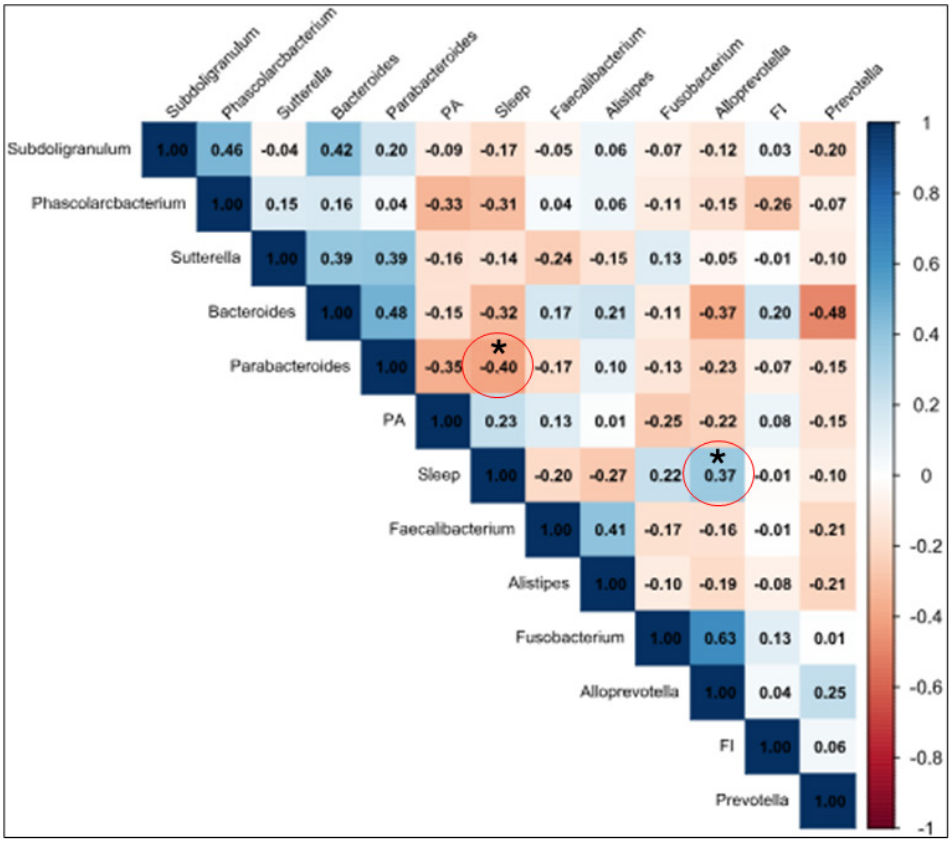

Figure 6: Heatmap using Pearson's correlation between gut microbiota and sleep, physical activity and fibre intake in BD, MDD and HC groups.

DISCUSSION

This study provides preliminary yet important evidence that the alteration in gut microbiome compostion may play a contributory role in the pathophysiology of bipolar disorder (BD) and major depressive disorder (MDD), with additional links to lifestyle factors, particularly sleep duration. Key observations include: (1) the identification of distinct taxonomic differences at the order level, with Oscillospirales and Coriobacteriales enriched in healthy controls (HC), while Acidaminococcales were enriched in MDD, suggesting their potential as disorder-specific microbial signatures; and (2) the detection of significant correlations between sleep duration and the relative abundances of *Alloprevotella* and *Parabacteroides*. Notably, these taxa are involved in pathways related to inflammation and tryptophan metabolism, implicating a possible microbial influence on the gut-brain-sleep axis. The depletion of *Faecalibacterium* within the order Oscillospirales — a key butyrate-producing genus — in BD and MDD groups further underscores a potential loss of anti-inflammatory microbial activity in mood disorders. Collectively, these findings not only align with emerging evidence linking the gut microbiome to neuropsychiatric health but also provide a foundation for identifying microbial biomarkers and therapeutic targets for mood disorder management. Further mechanistic and longitudinal studies are warranted to validate these associations and explore causality.

At the phylum level, both BD and MDD groups exhibited higher Bacteroidota and lower Firmicutes abundance. Meanwhile at the genus level, *Bacteroides* were most abundant in MDD, *Prevotella* in BD, and *Faecalibacterium* in HC. Taxonomically, *Bacteroides* and *Prevotella* belong to the Bacteroidota phylum and are Gram-negative bacteria, while *Faecalibacterium* is a Gram-positive bacterium from the Firmicutes phylum (29, 30). The clinical significance of these bacteria varies: *Bacteroides* has been consistently associated with affective disorders and negative mood, potentially due to its opportunistic pathogenic nature while the role of *Prevotella* in depression remains inconclusive, with studies showing both positive and negative health outcomes (2, 29). In contrast, *Faecalibacterium* has shown a consistent negative association with both depression and BD across multiple studies (2).

These findings align with several previous studies as well in this study. For instance, Hu et al. found that MDD patients had higher levels of *Bacteroides* and lower levels of *Faecalibacterium* compared to HC (31). Similarly, Zhang et al. reported higher *Prevotella* levels in BD patients with an overweight constitution, which aligns with the BMI of BD participants in this study (32). However, Chen et al. reported contrasting findings, observing lower *Prevotella* levels in BD compared to HC (33). Therefore, the alteration of these microbiota, especially low *Faecalibacterium*, may contributes to

the pathogenesis of BD and MDD attributing to their well-known beneficial towards humans' health such as synthesising the needed SCFA.

Additionally, to further distinguish between BD, MDD, and HC using specific gut microbiota or biomarker discovery, a LEfSe analysis was conducted. Based on the ASVs, three bacterial taxa at the order level were identified with Oscillospirales and Coriobacteriales enriched in HC and Acidaminococcales in MDD, suggesting these as potential disorder-specific markers. In the Oscillospirales order, *Faecalibacterium*, *Subdoligranulum* and *Ruminococcus* are the genera found in this study. Among these three, only *Faecalibacterium* was abundant in HC compared to the BD and MDD, while *Subdoligranulum* was the most abundant in the BD group, whilst *Ruminococcus* in the MDD. On the contrary, Radjabzaedah et al., discovered elevated levels of *Subdoligranulum* in healthy individuals compared to depressive patients and cited many other studies aligned with their results (34). For *Ruminococcus*, studies have narrated elevated abundance of this genera in the HC compared to the BD and MDD, contradicting our findings where this genus is more abundant in both BD and MDD (35, 36). Nonetheless, the enrichment of Oscillospiralles in HC for this study may indicates their benefits towards gut-brain axis especially with their well-documented beneficial role such as butyrate producer and anti-inflammatory.

Whereas for Coriobacteriales, studies have reported that this taxon and its members at various levels, including family, genus and species, are elevated in depression patients (2, 35). However, Knuesel and Mohajeri also emphasised where this genus is associated with BD on remission and lower anhedonia state (2). Meanwhile in this study, Coriobacteriales was predominant in the HC compared to the BD, as indicated by the *Collinsella* abundance. Lastly, for Acidaminococcales, *Phascolarctobacterium* and *Acidaminococcus* were the genera found in this study. Although the LDA score showed their association with MDD, they were elevated in BD and HC, respectively. Therefore, while the findings for these genera with BD or MDD is limited or inconclusive, these findings suggest that specific gut microbiota signatures particularly *Collinsella* may serve as potential biomarkers distinguishing BD and MDD from the healthy individuals.

Nevertheless, the alpha and beta analyses in this study did not reveal significant differences across the groups, which is consistent with findings from reviews by Knuesel and Mohajeri, who discovered that both alpha and beta diversity in BD and MDD are mostly similar with HC (2). However, McGuiness reported a slightly different, noting that while there is similarity in alpha diversity, beta diversity shows a higher number of studies reporting dissimilarities between MDD and BD with HC (12). This suggests that while there is similarity in overall gut microbiota diversity, the specific gut microbiota differs between BD, MDD, and HC. This finding underscores the importance of examining microbial composition at finer taxonomic resolutions, as broad diversity metrics may not capture the subtle changes in the gut microbiota composition.

Correlation study between identified microbiota and lifestyle factors enables further understanding of the environmental factors contributing to BD and MDD development. Sleep disorders are a common issue, with 70% and 92% of BD and MDD patients, respectively, experiencing them (37). In this study, the sleeping duration for BD and MDD is similar between short and long duration and not significantly differ with HC. Interestingly, based on the Pearson's correlation coefficient, sleep duration was the only lifestyle variable that has modest correlation with gut microbiota, specifically *Parabacteroides* and *Alloprevotella*. Sleep duration is negatively correlated with *Parabacteroides* and positively correlated with *Alloprevotella*.

Hypothetically, gut microbiota may modulate sleep through three mechanisms, which are; i) microbial metabolite with effects on sleep such as serotonin and melatonin, ii) indirectly affecting sleep by modulating the inflammatory responses and iii) alteration of gut microbiota composition through sleep and gene activity (38). Given the observed correlations between sleep duration with *Alloprevotella* and *Parabacteroides* abundance, monitoring these microbial taxa may offer complementary biomarkers to evaluate sleep-focused therapeutic interventions in BD and MDD management. While studies investigating the association between *Parabacteroides* and sleep in BD or MDD subjects are limited, previous studies suggest a link between *Parabacteroides* and sleep disturbances. Chen et al. reported an increased abundance of *Parabacteroides* in individuals with poor sleep quality following *Bifidobacterium* supplementation (39). Similarly, Zhang et al. found *Parabacteroides* to be enriched in sleep-deprived mice with anxiety-like behaviour, indicating a possible microbial response to sleep disturbances (40). This study observed a significantly weak negative correlation between *Parabacteroides* abundance and sleep duration, suggesting a low but notable association. Furthermore, Wang et al. predicted *Parabacteroides* to be involved in tryptophan metabolism, a key pathway influencing serotonin and melatonin levels, which are critical regulators of sleep and associated with depression (41). *Parabacteroides* has the ability to promote indole production instead of serotonin by influencing tryptophan metabolism through the released of tryptophanase (TnaA) (42). Taken together, these findings suggest that *Parabacteroides* may be involved in sleep disturbances through microbial shifts and neurochemical pathways, though further research is needed to elucidate its precise role.

Likewise, the association of *Alloprevotella* with sleep duration in BD and MDD remains underexplored, though emerging evidence links this genus to various sleep disorders. Mendelian Randomization analysis has identified a significant association between *Alloprevotella* and narcolepsy type 1 (NT1), suggesting a potential causal link between this genus and sleep health (43). In addition, *Alloprevotella* abundance has been reported to have a moderately positive association with chronic insomniac individuals (44). Conversely, Yao et al. and Wang et al. found that *Alloprevotella* abundance was significantly reduced in sleep-deprived healthy adults, reinforcing its involvement in sleep regulation (45, 46). Our study finding was consistent with Yao et al. and Wang et al., we observed positive association between sleep duration and *Alloprevotella*, suggesting their potential role in mental health. (43). Sleep quality may be associated with *Alloprevotella* via neuroinflammation by increasing the lipopoplysaccharides (LPS) levels, activation of toll-like receptor 4 (TLR-4)/transciption factor-kβ (NF-kβ) signalling pathways, increase in production of proinflammatory cytokines such as tumour necrosis factor-α (TNF- α), interleukin-1β (IL-1β), and IL-6, and reduction of anti-inflammatory cytokine IL-10 (46). As sleep disorder are common in both BD and MDD, these findings raise the possibility that gut dysbiosis may contribute to or exacerbate sleep-related issues in these mood disorders. Besides, poor sleep quality may also alter the gut microbiota, creating a bidirectional relationship that warrants further investigation.

A healthy and balanced diet is crucial in maintaining balance gut microbiota composition, as some of the prebiotics are strictly obtained from the diet, whereas an active lifestyle may promote the lactate cycle hence increasing the availability of SCFA for gut microbial activity which in turn can enhance human metabolic capacities and reduce inflammatory effects (47). Based on the physical activities assessment, both BD and MDD significantly spent lesser time compared to HC group. Meanwhile, the fibre intake for all groups is adequate. Contrary to expectations, fibre intake and physical activity did not show significant associations with gut microbiota composition in this study. This may be due to the small sample size or the homogeneity of the study population in terms of these lifestyle factors. Future studies with larger, diverse participants and additional analysis such as KEGG, to determine their mechanisms are needed to explore these relationships further, as both fibre intake and physical activity are known to influence gut microbiota which may improve the mood disorder symptoms (48).

Several notable limitations of this study warrant further examination. The primary limitation is the small sample size of each group, which may limit statistical power. Additionally, the fact that many BD and MDD patients were on medications could potentially influence gut microbiota diversity measures (49). Moreover, while 16S rRNA sequencing provides reliable genus-level classification, its inability to resolve up to species level and certain closely related taxa may have contributed to the observed lack of significant diversity differences. Our use of LEfSe for biomarker identification has inherent constrains as it cannot adjust for confounders and relies on univariate testing. Confounders such as strain specificity, geographical differences, dietary habits, ethnicity and variations in microbial profiling protocols may have led to contradict previous studies (50). Therefore, future investigations employing multivariate methods like MaAsLin2 coupled with core microbiome analysis could provide more robust and reproducible microbial signatures. The current findings could be expanded in future work by integrating multi-omics approaches, including metabolomics to assess microbial-derived metabolites and targeted assays for inflammatory and neuroendocrine markers. This approach will provide deeper mechanistic insights into the link between *Alloprevotella*, neuroinflammation, trytophan metabolism and sleep quality in BD and MDD patients. Lastly, this work is a cross-sectional study, it lacks the ability to establish causality between gut dysbiosis and lifestyle factors in BD and MDD patients. Therefore, a larger sample size, better controlled confounders and more robust analytical method could facilitate a more meaningful comparison and help determine the clinical applicability of the findings.

CONCLUSION

In conclusion, this study supports the presence of gut dysbiosis in individuals with BD and MDD compared to healthy controls. Oscillospirales and Coriobacteriales, together with Acidaminococcales, were distinctly abundant in HC and MDD, respectively. Furthermore, the sleep duration shows a modest correlation with *Parabacteroides* and *Alloprevotella*. These findings suggest a potential link between the gut-brain axis and sleep regulation in mood disorders. Understanding the role of gut dysbiosis in BD and MDD and their association with lifestyles could offer alternative therapeutic interventions, such as sleep therapy, probiotics, prebiotics, or dietary modifications, aimed at restoring gut microbial balance and improving mental health outcomes. Nevertheless, further research is needed, taking into account various influencing factors to enhance the study results. It is suggested that alternative approaches, including advanced multi-omics techniques such as transcriptomics, proteomics, and metabolomics, be utilised. Ideally, longitudinal studies should be incorporated in future study as it enables to determine whether gut dysbiosis is a cause or consequence of BD and MDD and to explore the temporal relationships between gut microbiota, lifestyle factors, and psychiatric symptoms.

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